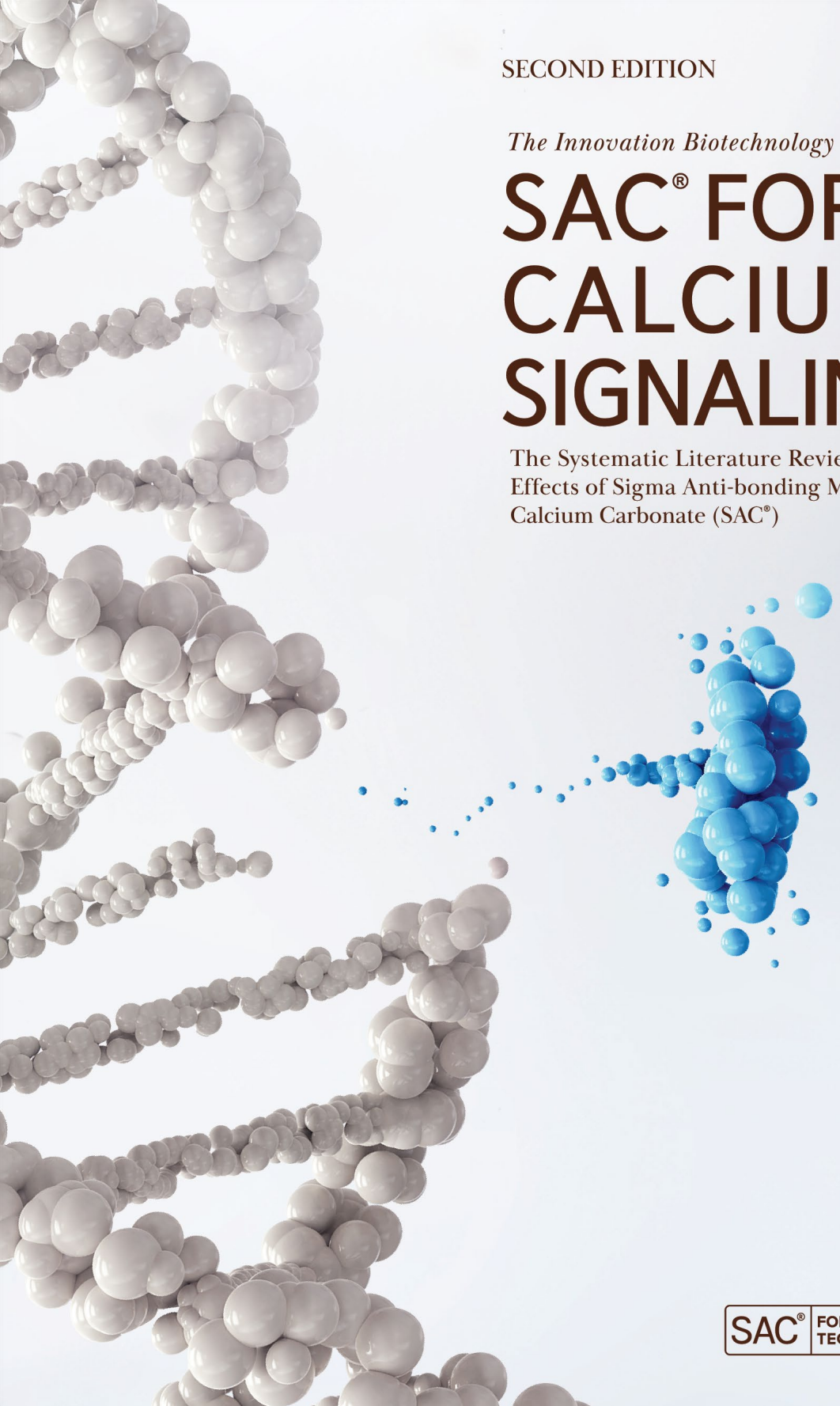
SECOND EDITION

The Innovation Biotechnology

SAC® FOR CALCIUM SIGNALING

The Systematic Literature Review for the Effects of Sigma Anti-bonding Molecule Calcium Carbonate (SAC®)



SAC® FORMULATION TECHNOLOGY

SAC® FOR CALCIUM SIGNALING

The Systematic Literature Review for the Effects of Sigma Anti-bonding Molecule Calcium Carbonate (SAC®)

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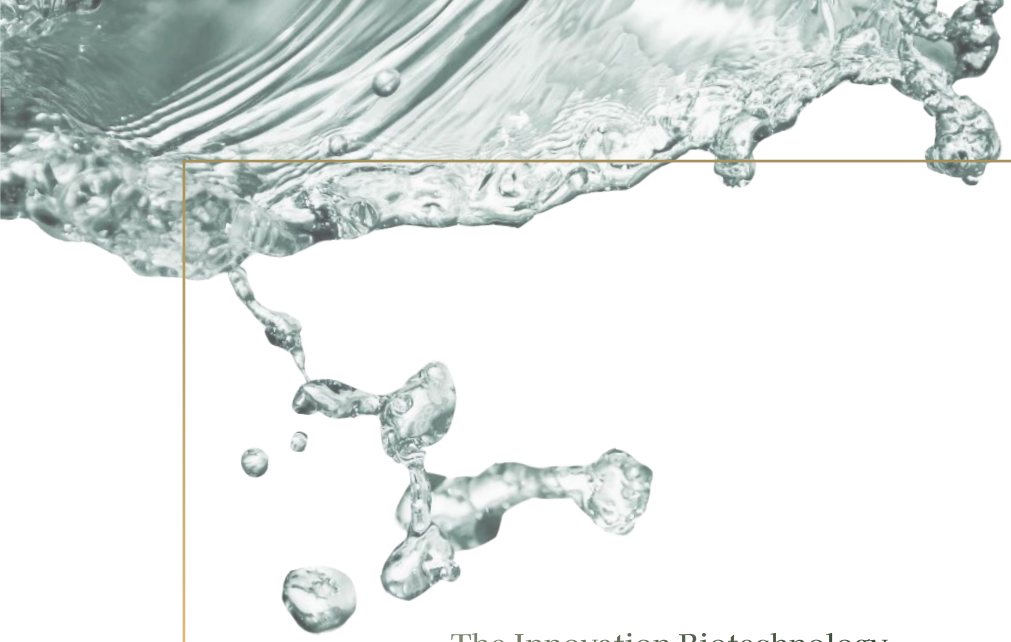
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The Innovation Biotechnology

SAC®

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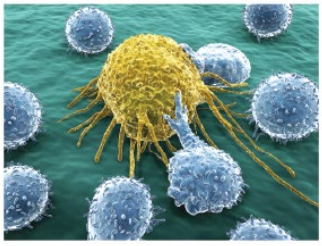
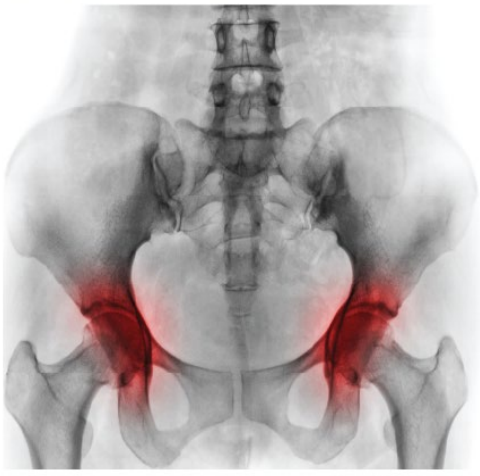
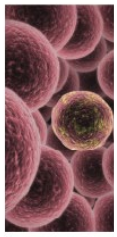
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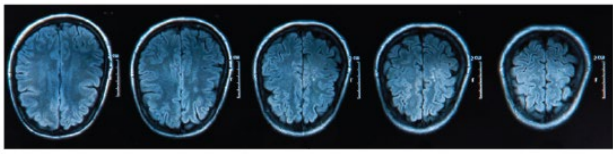
The Systematic Literature Review
for the Effects of Sigma Anti-bonding
Molecule Calcium Carbonate (SAC®)

CBHI
Calcium & Bone Health Institute

Introduction to CBHI Canada



The Calcium and Bone Health Institute of Canada (CBHI Canada) is a scientific-research-based, nonprofit organization that was founded in 2001.

Our research is primarily focused on the role that calcium plays in our body, and in addition, we've invented a novel form of calcium, called Sigma Anti-bonding Molecule Calcium Carbonate ($CaCO_3$). As it is used as a physiologically-active plasma-ionized calcium in our body, which enhances calcium homeostasis as well as the influx and efflux of intracellular calcium ions, it can help our body maintain its optimal status. Moreover, as the appropriate influx and efflux of calcium ions modifies mitochondrial function, it also improves protein synthesis, which can be the key to treating chronic degenerative diseases, including cancer.

To further our research, we have collaborated with numerous respected researchers from many different disciplines. These researchers come from institutions such as UAMS, SFU, UBC, UC Davis, Hallym Univ. Korea, TCM China, NSERC Canada and the Government of British Columbia (BC). We also collaborate with many other research centers to combine our research with fundamental sciences such as mathematics, chemistry, physics, and biology. Moreover, we work with many applied science faculties, such as engineering and computing science faculties, to enhance the depth of the research.

The Calcium and Bone Health Institute of Canada (CBHI Canada) is one of the few Canadian research-based institutes that was founded specifically to perform research into calcium-related chronic diseases, such as cancer and osteoporosis, among others. By conducting laboratory and clinical research, we are endeavoring to find effective prevention and treatment methods. Moreover, through collaborative research with many different medical and pharmaceutical companies, we are proving the efficacy of Sigma Anti-bonding Calcium Carbonate ($CaCO_3$).

The mission of CBHI Canada is to provide better care for cancer, osteoporosis and arthritis patients by developing innovative and effective cancer, osteoporosis and arthritis treatments and to contribute to lower health care costs associated with cancer, osteoporosis and calcium-related disease.

Moreover, as osteoporosis has one of the highest global burdens among diseases, especially for women who experience it post-menopause, we are looking for ways to decrease costs associated with subsequent fractures caused by osteoporosis. Since 2011, we have been providing comprehensive osteoporosis treatment using SAC products. As a result, more

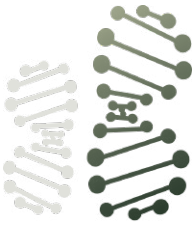
than 110,000 people worldwide have experienced the efficacy and effectiveness of SAC.

Furthermore, CBHI has developed AIC formulation technology, high purity AIC (Anti-orbital Ionic Calcium) based on SAC. AIC is being used for medical purposes only as AIC Therapy.

The CBHI is located in Coquitlam, BC, Canada. We are committed to discovering the causes of and cures for calcium-related diseases and believe this will be accomplished through research. By working together, we combine our innovative expertise to investigate questions related to all respects of bone and joint health.

"CBHI Canada envisions a world without cancer,
osteoporosis and any calcium-related diseases.
Our mission is to achieve this vision through a variety of profound research and clinical trials."

Paul. K. Lee



2nd Edition

Systematic Literature Review for the Effects of Sigma Anti-bonding Molecule Calcium Carbonate (SAC)

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Preface

Tremendous medical advancements have marked the 21st century, and these advancements have extended the life expectancy of humans farther than ever before. However, the progress of advancements in bone-related and cancer-related disease seems to be at a comparative standstill. In the majority of developed countries, the increase in an aging population has resulted in a higher risk of death caused by bone fractures. As a side effect, there is an increased social and economic burden on the general population. Also, the substantial investment in anti-cancer remedies has not helped in slowing down the rising death rate of cancer patients. This hopeless situation only exasperates the doctors in charge and adds greater frustration to the families of the cancer patients.

Therefore, at CBHI Canada, realizing the significant role of calcium in our health from the cellular level up, we have continued to research and develop ways to supply calcium ions into cells through passive transport. In March of 2009, CBHI was successfully developing SAC Calcium. In December of 2011, a clinical test in rats with osteoporosis proved successful in fully reversing the osteoporosis. CBHI then continued to treat osteoporosis patients across the spectrums of age, gender and race, proving the efficacy of this treatment. To date, many osteoporosis treatments have been developed to treat osteoporosis patients, including bisphosphonates, SERMs, teriparatide, and calcitonin, but none of them have been successful in curing osteoporosis.

SAC Calcium proves that even in the absence of Vitamin D, it is able to permeate the cell membrane and is absorbed by means of diffusion. Additionally, the small quantity of ionic calcium consumed has the effect of physiological plasma calcium. Therefore, with SAC Calcium, CBHI was able to effectively treat diseases such as cancer, diabetes, and osteoporosis, which are related to the lack of ionic calcium in the blood and cells. SAC Calcium provides promising remedies for different types of cancers including multiple myeloma, brain tumors, colon cancer, breast cancer, and leukemia.

In November of 2013, news reached us from S. Korea that Ms. Molly Holt (Age 79, Female) was receiving treatment for multiple myeloma. Since all the chemotherapy and radiotherapy received had proven ineffective

for her, she was ready to call all her relatives from the United States to say her last goodbyes. Without delay, CBHI sent SAC Calcium to Ms. Holt, and her condition significantly improved following treatment. Ms. Holt and her friends later visited CBHI and formally presented me with a plaque expressing appreciation, sincerely thanking me.

Both carcinomas and sarcomas have a very close relation to calcium ions. According to various forms of research, when ionic calcium levels decrease within the cell, the performance of the mitochondria, DNA, p53 protein, POT1 protein, NK-kB protein, cytotoxic T-lymphocytes and all stem cells subsequently decrease and can result in DNA mutations, which then accumulate and turn into carcinoma or sarcoma.

Increasingly, more and more researchers are actively writing about and are proposing the importance of the role of calcium. For instance, a claim by the American Neurological Association, which states, "Calcium is a key to solving Alzheimer's Disease and Parkinson's Disease," tells us how crucial the role of the calcium ion is in our body. CBHI believes that if healthy calcium levels are maintained within all of our cells, we will be free from all degenerative diseases.

I hope SAC Calcium brings renewed hope for many who are suffering from cancers, osteoporosis, and chronic and degenerative diseases. The successful completion of this book has been made possible by the assistance of several people to whom I owe a debt of gratitude. I would like to thank all the members of the CBHI Canada research team, Angie Lee, Steve Choi, Ben Ryu, Anne Chung, Neia Shin, Peter Lee and Alex Lee who contributed to the publishing of this 2nd edition. In particular, I am also very grateful to Dr. Antonio Jimenez and Dr. Subrata Chakravarty of Hope4Cancer, who took part and wrote the foreword in this book.



Ph.D. in Chemistry,
Paul. K. Lee
February, 2025

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I. Foreword

In abundance, we can sometimes find scarcity.

This paradoxical statement summarizes the relationship between the human body and calcium utilization. After oxygen, silicon, aluminum, and iron, calcium is the most abundant element in earth's crust, making up 4.2 % of its total mass. However, despite its literal abundance everywhere, it is ironic that it is the deficiency of this mineral that can be a major cause of concern in a variety of chronic diseases, including cancer.

Calcium is the most abundant mineral in the human body, making up 1.5-2.0 % of our total body weight. Its sheer insolubility means that 99 % of this calcium is found in the body undissolved and uncharged, concentrated in the bones, a key element in giving our skeleton its rigid structure. However, a small and critical concentration of this calcium remains dissolved in the blood and cells in ionic form. Optimal ionic calcium levels are controlled by hormones and are vital for the bioregulation of many of our body's functions. An imbalance in these bioregulatory processes can result in a clinical deficiency of ionic calcium that can manifest in the onset and progression of serious chronic diseases.

Ionic calcium, along with other bioregulatory substances, is part of what I call the "biological terrain," part of "God's Master Plan" for the maintenance of our health and well-being. In the healthy body, these substances exist in an exquisite equilibrium with abundant, buffering fail safes to compensate for unexpected imbalances. But with the wear and tear caused by aging, environmental affronts, poor nutrition, lack of exercise and inadequate quality sleep, our bioregulatory systems slowly and steadily get thrown off balance.

For over 30 years now, I have treated thousands of cancer patients with integrative treatment protocols using my 7 Key Principles of Cancer Therapy™. I can literally say that almost every case of cancer I have treated has identifiable biological terrain problems. This is good news because it means that restoring the balance of the biological terrain can indeed put us back on track towards achieving our healing goals. Conversely, not paying attention to it means that we may end up simply treating the symptoms and not the underlying cause. Many of our integrative treatments

seek to restore the harmony lost at our three levels of existence: the body, the mind, and the spirit. The truth is that the biological terrain is too complex for any one treatment to repair. But I have found that nature has already built in all the necessary tools to assist the body in healing itself.

We simply need to stop abusing it and find the skills needed to unlock its limitless potential.

Restoring ionic calcium to its bioregulatory equilibrium is particularly difficult, given the inadequacy of available supplements in restoring calcium levels or their dangers in inducing conditions exacerbated by hypercalcemia. At any rate, supplementation does not address the root cause of the imbalance in the first place. That is why CBHI Canada, under Dr. Paul Lee's stewardship, has done the world a tremendous service by not only bringing its attention to the problems surrounding ionic calcium bioregulation, but also by proposing an elegant solution in the form of a major leap in treatment technology, known as SAC Therapy.

In its second edition, this book provides deep evidence-based insight into how SAC Therapy can help in restoring ionic calcium balance and thereby make an impact in the treatment of diverse chronic diseases including osteoporosis, type 2 diabetes, Alzheimer's disease, and cancer. I have used SAC Therapy extensively with my cancer patients as part of their personalized treatment protocols with remarkable results. That is why it gives me great pleasure to not only write this foreword and a contributory chapter for this book, but also to endorse the product itself for its remarkable benefits.

Dr. Antonio Jimenez, M.D., N.D.
Founder and Chief Medical Officer
Hope4Cancer Treatment Centers

HOPE 4 CANCER
TREATMENT CENTERS

II. Introduction

Through copious amounts of academic literature, books, and media, people have become well-informed that calcium is an essential mineral in maintaining our bone health. As there are many calcium supplements available in the market, calcium is heavily advertised for its positive effects on our bodies. As many pieces of research have shown, calcium plays a critical role in treating osteoporosis, which is one of the most prevalent aging-related chronic degenerative diseases we face. Moreover, as the demand for calcium is increasing, many medical scientists are giving attention to the effects of intracellular calcium ions on our health. While cellular health translates to our overall health, calcium is closely related to mitochondrial function, which is the key to treating aging-related chronic degenerative diseases as well as cancers. Nevertheless, even though it had already been established that balancing intracellular calcium ions is crucial for cellular health and immensely potent in the treatment of diseases that we have not yet eradicated, there were no particular calcium treatments that could directly affect cellular calcium homeostasis.

Through numerous published, academic literature sources, it has been proven that calcium plays a crucial role in signaling mesenchymal and embryonic stem cells, replicating DNA, activating mitochondrial respiration, and balancing blood pH levels. Although we already knew that restoring intracellular calcium ions is essential for mitochondrial health, there were no particular calcium supplements on the market that directly affected intracellular calcium ion levels. However, remarkable progress has been made by the Calcium & Bone Health Institute of Canada (CBHI) with the invention of a novel form of calcium carbonate (CaCO3) that has a unique bonding structure, and a significantly increased rate of calcium absorption as ionized plasma calcium. It directly affects extracellular and intracellular calcium ion levels, which modify mitochondrial function, offering effective treatment for chronic degenerative diseases. Existing as a physiologically-active ionic form of calcium carbonate in water, this novel calcium shows excellent efficacy in terms of absorption rate, solubility, and bonding structure as compared to the regular calcium carbonate currently on the market. We named it Sigma Anti-bonding

Molecule Calcium Carbonate (SAC).

While SAC provides physiologically-active ionic calcium carbonate that is directly bio-available, the demand for calcium ions in our bodies is always high, especially for those with active lifestyles. SAC acts directly as plasma-ionized calcium in the blood and cellular membrane as it is absorbed through the epithelial tissue of the stomach (mucosa). Moreover, because SAC uses a passive transport system, it is incorporated into our cellular membrane without the use of any biomechanical energy. Regular calcium carbonate, however, uses an energy-draining active transport system, wherein absorption through our cellular membrane is aided by vitamin D. As a result, SAC is much more efficient and beneficial in balancing the calcium homeostasis of our bodies.

It is recommended to ingest 5 mL of SAC mixed with 500 mL of water because it uses osmotic pressure for absorption into the cell. Moreover, since SAC incorporates weak sigma Anti-bonding, it is easily broken down in the stomach with the osmotic pressure and low pH conditions that exist therein. Due to the osmotic pressure and the reaction of the hydrochloric acid (HCl) in the stomach during chemical digestion, SAC is easily broken down in the stomach and absorbed directly through the mucosa.

As SAC is directly ionized in the cellular membrane, it also has excellent solubility, with an ionizing rate about 200 times greater than that of regular calcium carbonate. When a regular calcium supplement is taken, vitamin D is often recommended, because the role of vitamin D plays in helping calcium to bind to peptides and be transported to blood vessels. However, SAC bypasses all these processes by being directly absorbed through the cellular membrane in ionic form and is immediately bio-available. Regular calcium ends up as inactive protein-bound calcium instead.

Calcium exists in three different forms in serum plasma: 40-45 % protein-bound calcium, 5 % inorganic calcium, and 45-50 % ionized calcium. The only calcium that is physiologically active and triggers osteoblast (bone forming cells) and osteoclast (bone degrading cells) activity is ionized calcium. This physiologically-active calcium signals the pituitary gland to secrete pituitary hormones, which in turn triggers the release of thyroid hormone (TH) and parathyroid hormone (PTH). These hormones

stimulate the uptake of calcium in the bones by activating osteoblasts, which lead to the strengthening of bones. Moreover, it also triggers the activity of osteoclasts, so as to enhance the bone turnover rate to improve bone quality. SAC, which elevates the serum concentration of ionic calcium, plays a critical role in activating these hormones to protect our bone health.

As SAC participates in the activity of intracellular calcium influx and efflux, it normalizes mitochondrial function, which in turn can treat cancers and other chronic neurodegenerative diseases such as Alzheimer's disease, epilepsy, arrhythmia, mitochondrial dysfunction-related diseases, osteoporosis and type 2 diabetes. Furthermore, by balancing intracellular calcium influx and efflux, it can initiate the activity of mesenchymal and embryonic stem cells, thereby working to regenerate cellular functions and provide the key to treating aging-related chronic degenerative diseases.

III. SAC Calcium
(Sigma Anti-bonding Molecule Calcium Carbonate)

"Sigma Anti-bonding Molecule Calcium Carbonate (SAC): Physiological ionic calcium in serum uses a passive transport system when absorbed into the bloodstream via the epithelial cells of the stomach without vitamin D."

"Two years ago, in Tokyo, I invited 20 people who attended the seminar to participate by trying out SAC Calcium dilution water after the lecture. After a while, while I was setting up the ultrasonic bone density meter, a number of the participants who took SAC Calcium began to express that they were "feeling a strength that they had not felt before from their weak body." We believe that this was due to the fact that SAC Calcium quickly enters the bloodstream and cells, and causes the metabolism of mitochondria to begin to function normally "

1. Introduction

The bones are our largest reservoirs of calcium, containing about 99% of the calcium found in the body. Calcium in the form of calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is also found in the teeth, in the circulatory system, in the muscles, and in other tissues. But how much do we really know about the stuff?

Calcium plays a crucial role in the body. This vital mineral helps to regulate the blood vessels, oversees our hormone production, and is key to intracellular signaling the way the cells communicate. It's also involved in muscle contraction, nerve communication, and other functions.

What does this mean? First off, in the human body, calcium homeostasis and by extension, calcium resorption and bone formation is paramount to our overall well-being.

Second, we need to pay close attention to the factors that influence calcium absorption, bone formation, and homeostasis. These include

diet, exercise, vitamin D3, thyroid hormone (TH) and parathyroid hormone (PTH), and age. When these factors are out of balance, calcium homeostasis is affected and disease states such as calcification (the accumulation of excess calcium), osteoporosis, kidney damage, p53 dysfunction, the POT1 gene, and various cancers resulting from DNA mutation may occur.

Depending on the underlying abnormality, there are a number of treatments available. To understand them and the complexities surrounding homeostasis it's essential that we review the different levels of calcium, and the importance of homeostasis in our calcium absorption.

The Lowdown on Calcium Absorption

The human body generally meets its daily calcium needs through foods like eggs, milk, and other dairy products. Additional foods rich in calcium include sardines, fortified fruit juices, soybeans, and even dietary supplements.

Sounds like it's easy to maintain homeostasis, right? Not so fast. While our calcium consumption is important, a sufficient calcium intake won't do much without adequate vitamin D levels. These are what determine how much calcium the intestines actually absorb.

Now, where people above the age of 40 are concerned, several studies point to greater rates of calcium malabsorption generally associated with vitamin D deficiency. In postmenopausal women (coupled with the reduced calcium absorption we experience with advancing age), lower estrogen levels may also play a role. This too has been found to lead to a greater predisposition for osteoporosis.

But before we explore calcium absorption among different age groups, we should revisit calcium itself. The mineral exists in three different forms:

1. *Calcium salts (5-10 %)*
2. *Protein-bound calcium, mostly to albumin (40-45 %)*
3. *Free-circulating ionized calcium, at a concentration of about 1.25 mmol/L (50 %)*

With sufficient vitamin D levels, about one-third of our dietary calcium is absorbed in protein-bound form. In the absence of vitamin D, however, the unabsorbed calcium is excreted which can cause constipa-

tion, mineral imbalance, destruction of the gut microbiome, and various immune problems.

Excessive dietary calcium intake, especially with subpar absorption, can interfere with the absorption of other minerals like iron, magnesium, and zinc. It can also alter the intestinal flora, resulting in greater stomach acidity, a spike in bile acid and fatty acid precipitation, and an increase in colonic pH.

Strategies for Improving Calcium Absorption

What is a common factor affecting the body's calcium utilization? Exercise.

Physical activity can help to maintain bone mineral density (BMD), with high-intensity exercises, in particular, resulting in mechanical loading effects that can increase bone mineral deposition and density.

In short, getting enough exercise is critical. With adequate exercise, the complex protein-bound form of calcium dissociates to yield protein and ionized calcium, or the physiologically-active form of the mineral.

Conversely, when the protein component is metabolized, the physiologically-active (ionized) form of calcium takes on roles such as bone mineralization, maintenance of vascular tone, blood clotting, nerve communication, cell signal regulation (or, the communication between the cells and external stimuli), muscle contractions during exercise, and autonomous muscle contraction.

And here's the thing: In sedentary people, including those facing prolonged immobilization or weightlessness, some of this protein-bound calcium can accumulate in the body's tissues, causing calcification. This can be cause for concern when increasing your physical activity isn't an option.

Calcium supplements may well be the solution, as they bypass the need for exercise in a method known as passive absorption. SAC (Sigma Anti-bonding Molecule Calcium Carbonate) contains calcium that can easily dissociate into a free ionic state in the lumen of the stomach's epithelial cell calcium channels, as a free ionic calcium without vitamin D3 (1,25-dihydroxycholecalciferol).

This is an efficient way to mitigate calcium deficiency. That said,

calcium absorption and homeostasis even with adequate vitamin D levels and proper exercise tend to vary based on age.

Calcium Homeostasis in People Under 40

In this age group, calcium homeostasis is determined by the amount of calcium in circulation meaning whether there's enough calcium in the body.

Without enough ionized calcium, the parathyroid glands will release the parathyroid hormone, thereby promoting calcium reabsorption from the kidneys, activating the osteoclasts to release calcium from the bones into the circulatory system simultaneously converting vitamin D in the kidneys into its most bioactive form.

Vitamin D helps to enhance calcium absorption as protein-bound calcium from the intestines, all while elevating calcium absorption into the bones via osteoblast proliferation and activity.

With an excess of ionized calcium, the osteoblast cells will activate and facilitate calcium uptake into the bones. Additionally, the kidneys will activate less vitamin D, all while inhibiting parathyroid hormone secretion.

Ultimately, the thyroid will generally leverage vitamin D to achieve homeostasis in younger people. This can help to address calcium deficiency (or excess calcium levels) without the need for supplementation.

Calcium Homeostasis in the Post-Menopausal and the Elderly

Calcium homeostasis depends on thyroid and parathyroid hormone availability in this age group, as well as whether there is a deficiency or an excess of calcium in the body.

In the elderly, intestinal calcium absorption is often inadequate. In addition, an age-related hormonal decline may yield negative effects on bone health. Increasing evidence points to vitamin D deficiency, along with reduced physical activity, as the main causes of age-related calcium deficiency.

What happens in these states of calcium deficiency? The parathyroid hormone stimulates osteoclast cells, prompting the bones to release

calcium. Although this can initially help to restore calcium reserves to appropriate amounts, this often happens at the expense of bone loss leading to osteoporosis.

As such, osteoporosis is most common in postmenopausal women and elderly people. To help treat this condition, drugs like bisphosphonates can inhibit the activity of osteoclast cells associated with parathyroid hormone release, inactivating the osteoclasts and subsequently ceasing all calcium ion mobilization within the bone matrix. This can also mitigate calcium deficiency in the serum.

Alternatively, older populations may face low calcium concentrations alongside deficiencies in parathyroid hormone production in hypothyroid states. Due to problems involving parathyroid hormone secretion, the body might not stimulate enough, or any, osteoclast activation and because ionic calcium is not released from the bones in such conditions, a calcium deficient state may ensue. This generally involves symptoms of osteomalacia (or weak bones due to lack of calcium), tetany, hypotension, and issues with cardiac rhythm.

To rectify hypocalcaemia, levothyroxine can stimulate the release of the parathyroid hormone. This will help activate the osteoclasts, which can then release calcium from the bones into the circulatory system, simultaneously resulting in bone loss (osteoporosis).

Finally, there could also be an excess of circulating calcium in the elderly, which inhibits the production of the parathyroid hormone via a negative feedback loop. However, since osteoblasts are largely inactive in this population, they are unable to deposit the excess calcium into the bones. The result is an abnormal accumulation of calcium in vital organs such as the kidneys, the heart, and the blood vessels, potentially leading to organ damage.

Calcium Absorption and Homeostasis: Key Takeaways

Dietary calcium may be sufficient for younger people. Older individuals, however, need ample vitamin D to optimize absorption. In people above the age of 50, calcium supplementation is recommended.

Concurrently, exercise is crucial to calcium absorption and homeostasis, as it plays a key role in the dissociation of calcium from protein into

an ionized or physiologically-active form.

Depending on age, the body can remove excessive calcium from the bloodstream or release calcium from the bones to compensate for any insufficiencies. Without doing so, calcium accumulation can lead to organ calcification, or, conversely, to a state of calcium deficiency that would require supplementation.

Problems may also arise when the body releases too much calcium from the bones, a set of circumstances that would likely call for bisphosphonates, denosumab, etc., which could potentially be risky. Meanwhile, shortfalls in parathyroid hormone secretion can be treated using levothyroxine, which stimulates PTH production and osteoclast activation, releasing calcium from the bone in an effort to reach an equilibrium.

In closing, to fully reap the benefits of calcium, novel supplements, such as SAC, which provide calcium in its only physiologically-active (or ionic) form are beneficial. They not only guarantee increased calcium bioavailability, but they are able to go beyond addressing the underlying causes of calcium deficiency, activating ionic-calcium-sensitive physiological responses that can help to restore normal calcium levels.

2. Calcium Signaling

Calcium (Ca^{2+}) ions are the main communication signal between cells in intracellular and external fluids. After entering the cytoplasm, they have the effect of allosteric regulatory proteins on various enzymes and proteins, and play an important role in cell signal transmission. The secondary signal transmission caused by the activation of ion channels or indirect signal transmission channels such as G protein-coupled receptors is used for the information exchanged between cells and genes.

Many scholarly papers have pointed out that the intracellular concentration of calcium ions is generally maintained at about 100 nM, which is 20,000 to 100,000 times lower than the extracellular concentration. In order to maintain this low concentration in the cell, calcium ions flow from the cytoplasm into the extracellular space, the endoplasmic reticulum (ER), and occasionally into the mitochondria. Specific proteins in the cytoplasm and cell organs combine with calcium ions to act as buffers. A signal is generated when the cell is stimulated to release calcium

ions from the intracellular reservoir, or when calcium ions enter the cell through ion channels in the cell membrane.

Calcium is a ubiquitous secondary messenger with a wide range of physiological functions, including muscle contraction, neurotransmission of excitatory synapses, cell motility (including flagella and ciliary movement), fertilization, cell growth or proliferation, and neural procedural memory, external and internal memory such as production, synaptic plasticity and saliva secretion. In addition, the high concentration of calcium ions in cytoplasm, by means of calcification, can also cause cell inflammation and cell death, and play a role in reducing intracellular calcium concentration. With a decrease in intracellular calcium concentration, other calcium-related biochemical effects are produced during DNA replication, including the regulation of enzyme activity, ion channel permeability, ion pump activity, and cellular skeleton components.

The released calcium ions bind to and activate the protein-regulating calmodulin (CaM) to produce a high concentration of calcium ions. Calmodulin activates the calmodulin-dependent protein-kinase of calcium ions, or directly acts on other related proteins . In addition to calmodulin, there are many other calcium-binding proteins that regulate the biological effects of calcium ions.

In neurons, the accumulation of calcium ions in cytosol (cytoplasmic matrix) and mitochondria (mitochondrion), together with mitochondrial energy metabolism, is very important for the synchronization of neuronal electrical activities. Mitochondrial matrix calcium ions can reach tens of micromoles, which is necessary for the activation of isocitrate dehydrogenase (IDH), one of the main regulatory enzymes of the Krebs cycle.

In neurons, the endoplasmic reticulum (ER) can exist in a binary membrane system together with the plasma membrane, which can be used to integrate countless extracellular and intracellular signals in a network. This association with the plasma membrane yields new information about the endoplasmic reticulum and neurons in the nerve cell, which also serves in the formation of the structural characteristics of the endoplasmic reticulum, the ability to excrete calcium ions, and the release of specific calcium ions. The role of the protein system. In any event, when the calcium signal is activated improperly, cell metabolism will become abnormal, or mutate, and become the precursor of disease.

3. Calcium and Minerals (Macro, Micro and Trace Minerals)

There are more than fifty kinds of elements that make up our human body, including calcium, iron, carbon, hydrogen, nitrogen, and zinc. There are less than twenty kinds of elements with functions that are correctly understood by people. These elements interact directly and indirectly to participate in cell metabolism, with positive and negative effects. Even the lack of one of them may induce disease, and when the element is continuously lacking, it will become the cause of chronic disease.

More than a hundred years ago, ginseng cultivated with no pesticides and no fertilizers contained 20-30 kinds of minerals, which became a very important source of mineral intake for elements that were otherwise in short supply. In addition, although the composition of Chinese medicinal materials widely used in Chinese medicine contain the ingredients of Chinese medicine, the various minerals contained in the fruits, leaves, skin and roots of various Chinese medicinal materials are very effective for the treatment of diseases caused by a lack of minerals. They are thus effective and help in maintaining health.

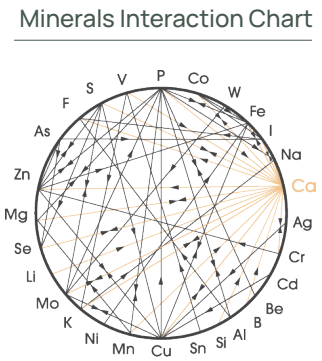


Figure 1. Relationship with Calcium

Calcium has a direct or indirect relationship with all the elements in the human body. According to the interaction of these elements, the metabolism from DNA replication in cell-to-cell division must be smooth to maintain the health of the body. Even if one is missing, problems will occur.

Element name	Function
Magnesium (Mg)	Strengthens the functions of the liver, kidneys and pancreas, so as to detoxify, regulate excretion and sugar content, regulate the body, regulate the production of human enzymes, regulate hemoglobin function, carbohydrate catabolism, build elastic muscles, regulate body shape and maintain balance.
Vanadium (V)	Regulates growth and reproduction. It is especially important for maintaining the health of bones, cartilage and teeth. It strengthens nerve cells, oxidizes organic substances with oxygen, provides natural radiation therapy, helps fat metabolism, hematopoiesis, hemostasis, strengthens peripheral blood vessels, and strengthens the heart.
Barium (Ba)	As a stable isotope mixture, it complements the metabolism of various substances in the cell. It is an essential mineral element for the human body and an element that helps calcium.
Selenium (Se)	Protects chromosomes from damage, promotes DNA repair, has an antioxidant capacity 2000 times stronger than vitamin E, improves immunity, has a synergistic effect on cancer chemotherapy and prolongs life, facilitates thyroid hormone metabolism, heavy metal detoxification, antioxidants, maintenance of cell membranes, inhibition and prevents the occurrence and metastasis of selenium-related cancer, having an anti-cancer effect.
Zinc (Zn)	Promotes growth and development, increases immunity.
Iodine (I)	Regulates thyroid function.
Phosphorus (P)	Formation of bones and teeth, maintains acid-base balance.
Chromium (Cr)	Normalizes insulin action, lowers cholesterol, improves and prevents arteriosclerosis.
Copper (Cu)	Hemoglobin production.
Boron (B)	Promotes absorption, helps bone growth, improves brain function.
Sodium (Na)	Regulates blood pressure, prevents edema, kidney disease, heart disease, prevents gastric ulcers and muscle disease.
Chlorine (Cl)	Improves digestive disorders.
Silicon (Si)	Prevents osteoporosis and prevents aging.
Sulfur (S)	Prevents hair loss, improves skin diseases, maintains healthy bones and cartilage.

Element name	Function
Titanium (TI)	Promotes metabolism and prevents cell aging.
Iron (Fe)	A hemoglobin component, important to oxygen supply.

Table 1. The Functions of Various Minerals in the Human Body

4. Calcium Signaling and the Calcium Channel

The regular flow of calcium ions in and out of cells is very important for cell metabolism. If calcium ions are too few or too many, problems will occur in the cells. The inflow and outflow of calcium ions are related to voltage, sodium, and potassium ions. Although no clear mechanism has been identified to date, researchers have given attention to and studied this field with interest.

In various clinical trials of SAC Calcium, it has been found that as mitochondrial function returns to normal, body temperature can rise, mesenchymal stem cells (MSCs) are activated, and gums are restored. It can be seen that SAC Calcium is a normal calcium signal, which can be presumed to flow into and out of cells normally through calcium channels.

5. What Is a Calcium Ion?

The calcium atom has 20 electrons, with 2 electrons in the outermost orbit. The moment when these 2 electrons are lost is called “ionization.” If two electrons are given to other atoms to complete the reaction, they are no longer ions. At this time, calcium functions as a cation and becomes an ion-binding substance. It is almost impossible to maintain calcium chloride and calcium sulfate in ion form without energy. The existence of ionized calcium in our body is due to the presence of energy. In this regard, although SAC Calcium exists in a general calcium molecular form, it is quickly ionized and absorbed after entering the stomach and contacting the mucous membrane.

6. Ionization, Solubility and the Bonding Structure

SAC has a unique chemical bonding structure as compared to other calcium carbonate ($CaCO_3$) products that are sold in the market as dietary supplements. Although the complete name of SAC is Sigma Anti-bonding Molecule Calcium Carbonate, its distinctive feature lies in its bonding strength. It is bonded with anti-sigma bonds, which are relatively weaker than regular ionic bonds. As it is weakly bonded, it is easily broken down when it is ingested orally along with an adequate amount of water. By means of osmotic pressure, it can be passively transported to the blood vessel system through the stomach lining. As other calcium supplements cannot be absorbed in the stomach due to the nature of their ionic bonding structure, they have to be absorbed in the duodenum with the aid of vitamin D and, with the aid of a protein transporter, transported into serum as a protein-bound calcium.

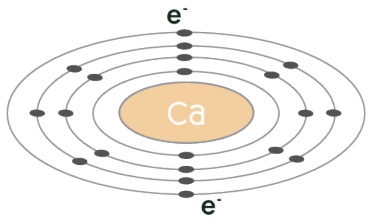


Figure 2. The Calcium Atom (The nucleus and electrons of calcium: Calcium atoms have a nucleus and 20 electrons surrounding the nucleus, where through the loss of two electrons on the outermost orbital they act as cations.)



Figure 3. A Comparison of Bonding Structures between SAC and Regular Calcium Carbonate

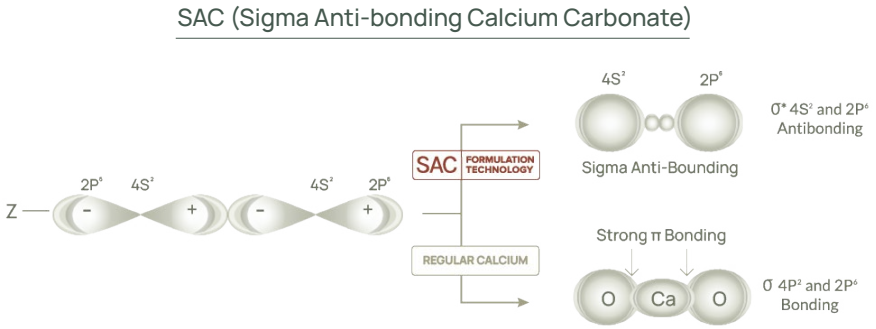


Figure 4. The Sigma Anti-bonding Structure of SAC (Sigma Anti-bonding Calcium Carbonate)

7. What is SAC formulation Technology?

SAC® Formulation Technology, was developed by CBHI in Canada in 2010. This innovative physicochemical method enhances material functionality through a unique interaction at the molecular level. SAC® is produced by leveraging the partial negative charge ($-\delta$ polarity) of oxygen in water molecules and the partial positive charge ($+\delta$ polarity) of calcium in calcium carbonate, creating a Sigma Anti-Bonding Molecule with a weak bonding force. This unique property allows SAC® to dissolve rapidly and be more readily absorbed into cells, generating active calcium ions (Ca2+) in the body. SAC® effectively reaches the threshold level of calcium-ion concentration in the blood to trigger a cascade of hormonal responses that stimulate bone-forming cells (osteoblasts) and enhance metabolic function. As the first calcium-ion delivery system of its kind, SAC® awakens the body's natural healing mechanisms, supporting not only a healthier bone structure but also revitalizing overall wellness from within.

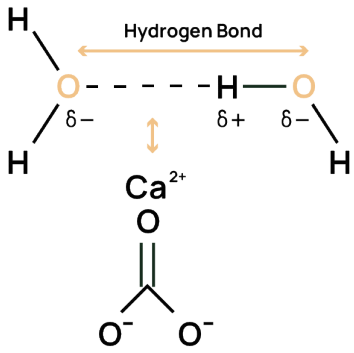


Figure 5. Calcium Carbonate Reacts with Water Molecules and by means of Osmotic Pressure, a Calcium Ion is Separated

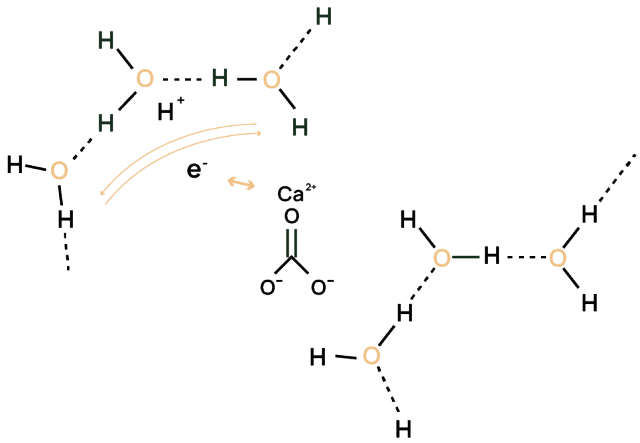


Figure 6. Water Hydrogen Bond 'Wires' Cooperativity and SAC; a Molecular Physical Phenomenon **Model**

SAC's formulation technology is ideal for stabilizing SAC Calcium in an unstable state, doing so by means of hydrogen bonding with the hydrogen atoms of water, stabilizing it in an aqueous solution, making it easily diffusible in mucous membranes for transport into cells and blood. This is atom-molecule bonding technology.



Figure 7. After the lid is opened in a state where the SAC Calcium and water molecules are stably combined, as the water molecules evaporate, SAC Calcium also evaporates together with water molecules and combines with carbon dioxide in the air to crystallize

Moreover, as SAC has a much lower Ksp coefficient (Ksp = 7.5×10-7 to 8.7×10-7 at 25 °C) than other regular calcium carbonates (Ksp = 3.7×10-9 to 8.7×10-9 at 25 °C), it is more readily soluble in water, which means that it can be more easily ionized in water. While they are easily ionized in water, calcium ions can be physiologically used in our body; and they eventually activate the calcium signals in the intracellular membrane. As more calcium ions are refluxed into the intracellular membrane, calcium ions start to oscillate and enhance our body's metabolism.

Activating calcium signals is crucial, as an increase in calcium ions initiates calcium signals that can modify protein synthesis by repairing mitochondrial activity; thereby strengthening our bone health and immune system. Moreover, as calcium balances lactic acid and ammonia in the cell, it can prevent neurodegenerative diseases.

Also, as calcium normalizes mitochondrial function, it can maintain our body temperature, blood pH, and oxygen levels.

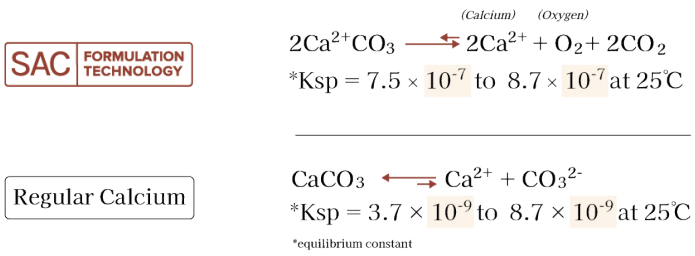


Figure 8. A Solubility and Ionization Comparison between SAC and Regular Calcium Carbonate

8. A Comparison of Chemical Reaction Rates between SAC and Regular Calcium Carbonate

Experimental value when reacting with acetic acid

$$CaCO_3 + 2CH_3COOH \rightarrow Ca(CH_3COO)_2 + CO_2 + H_2O$$

The reaction rate (r) measures the amount of reduction of calcium ions per hour; the chemical reaction formula is as follows:

$$-r_{Ca} = -\frac{d(Ca)}{dt} = \frac{k1\,(Ca)(ACT)^{1/2}}{k2 + (ACT\,Ca)/(ACT)}$$

$$-\int_{Ca=zero}^{Ca} \frac{d(Ca)}{d(Ca=zero)} = k\int_{0}^{t} dt$$

ACT: Acetate

Figure 9. The Amount of Reduction of Calcium Ions Per Hour

Time versus concentration run

Time (Sec)	Regular $CaCO_3$ [Ca] = [ACT]		SAC $CaCO_3$ [Ca] = [ACT]	
	mole/liter	1/[Ca]	mole/liter	1/[Ca]
0	0.2250	4.4440	0.2250	4.4440
15	0.2210	4.5240	0.1898	5.2690
30	0.2110	4.7390	0.1230	8.1300
45	0.2080	4.8070	0.0967	10.3410
60	0.1900	5.0760	0.0652	15.3370

Table 2. A Comparison of Reaction Rates between Regular Calcium Carbonate and SAC Calcium Carbonate

Based on the data above, when comparing the reaction rate between regular calcium carbonate and SAC Calcium carbonate, SAC Calcium carbonate showed a chemical reaction rate that was 3 times faster.

9. SAC Calcium Ions

"A SAC Calcium solution containing 5 mg (5 mL) of SAC Calcium diluted in 500 mL of water can instantaneously provide the human body with 1.25×10^{19} of ionized calcium."

The amount of physiological ionized calcium in the human body is about 5×10^{20}, of which 2.5×10^{16} is present in the cell. These intracellular calcium ions are used to initiate functions from heart muscle exercise to various metabolic functions, genetic replication, immune cell activity and other fields. When the amount is insufficient, problems begin to occur.

In addition, calcification occurs through an excessive flow of ionized calcium out of the bones and an excessive intake of protein-bound calcium. In particular, as postmenopausal women increase in age, calcium will decrease at varying rates. The resulting, associated diseases include cancer, diabetes, hypertension and other chronic diseases, intractable diseases, brain diseases, etc; with the total number of potential direct or indirect diseases reaching 150-200. SAC Calcium was developed to adjust ionized calcium levels to normal levels.

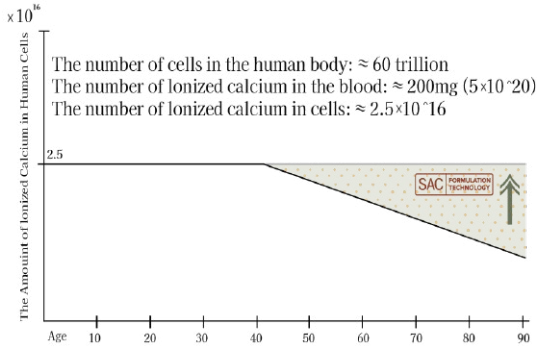


Figure 10. The Amount of Ionized Calcium in Human Cells at Different Ages

After 5 mg of SAC Calcium is absorbed into the human body, the number of physiological calcium ions is increased by 1.25×1019. When these physiological calcium ions flow into the blood and cells, and act as calcium regulating, calcium signaling, nerve and information transmission

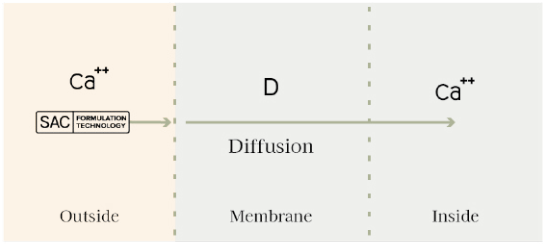
substances, all cells, such as hormones, enzymes, mitochondria, stem cells that were previously inactivated by the decrease in calcium concentration, begin to play a normal metabolic role.

10. SAC and the Absorption Route

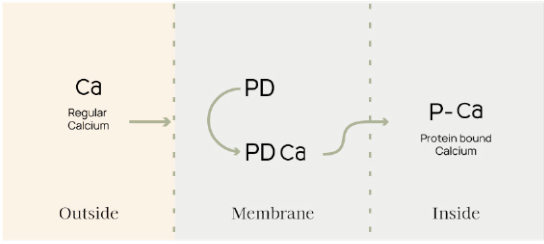
Most of the calcium that we require is supplemented by the foods we consume. However, due to lifestyle and dietary changes,

"An estimated 126.6 million Americans were living with a musculoskeletal disorder [that is mainly caused by a lack of calcium intake] in 2012." ("One in Two Americans Have a Musculoskeletal Condition, Costing an Estimated $213 Billion Each Year in Treatment and Lost Wages", 2016) To supplement the lack of calcium, people turn to nutritional supplements. Despite such efforts, the regular calcium supplements in the market are not physiologically-active ionic calcium. Although only a small amount of ionic calcium is needed for its physiological effects, the daily recommended intake of calcium is much higher because of its absorption efficiency.

The most prominent advantage of SAC over regular calcium supplements in the market is that SAC has an absorption rate that is 200 times more efficient in comparison to other supplements.



‹SAC Calcium Passive Diffusion Transport Mechanism›



‹Calcium Active Transport Mechanism›

Figure 11. The Use of Protein Transports for SAC and Regular Calcium Carbonate

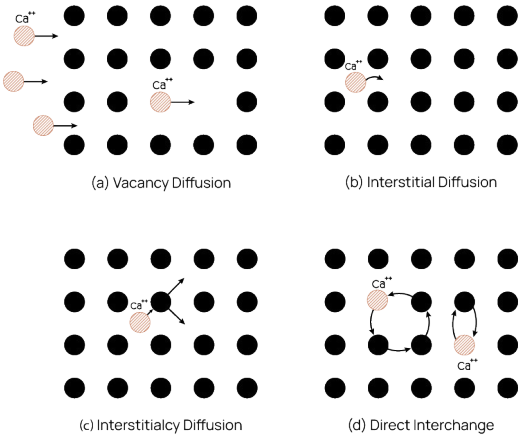


Figure 12. SAC Calcium Passive Transport Mechanism (Diffusion)

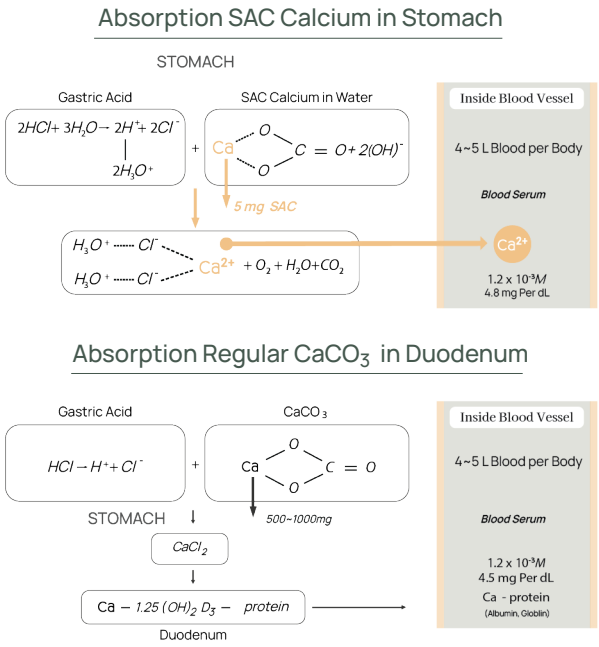


Figure 13. The Absorption Process of Passive Diffusion and the Active Transport System for SAC and Regular Calcium

It is prevailing knowledge that calcium is absorbed in the epithelial tissue of the jejunum, duodenum, and the small intestine with the aid of other biochemical energy. (Bronner, 2003) In its protein-bound form, calcium requires vitamin D to be absorbed into the epithelial tissue and requires protein porters to be transported into blood vessels and cells. However, SAC uses a passive transport system that does not require any biochemical energy for absorption. Moreover, since it is diffused into the blood vessels through the epithelial tissue in the stomach (mucosa), it can be directly used as ionic calcium to trigger the hormone regulation involved in calcium homeostasis.

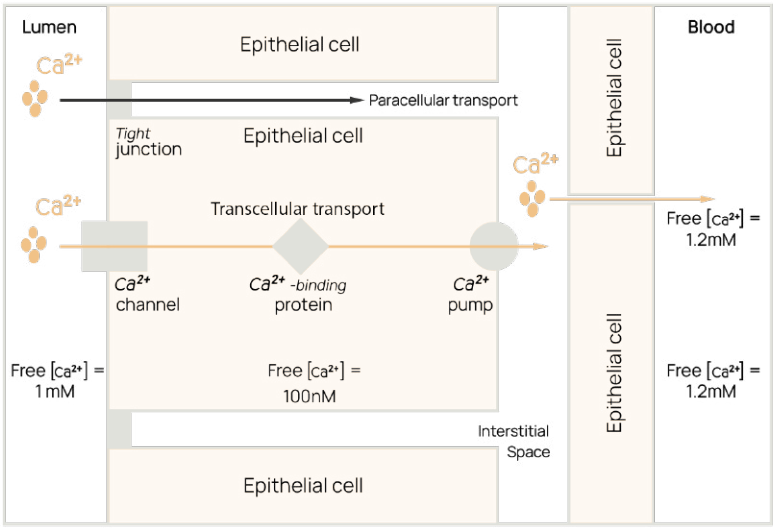


Figure 14. The Passive Transport Mechanism through the Epithelial Cell to the Blood Vessel

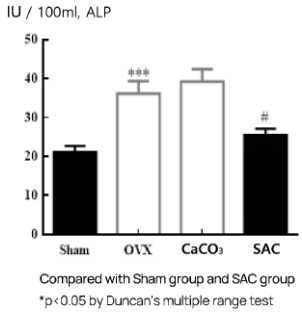


Figure 15. Effects of SAC and Calcium Carbonate for 4 weeks on ALP as a bone turn-over biomarker of ovariectomized rats. by TCM 301-307

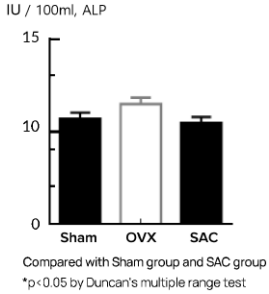
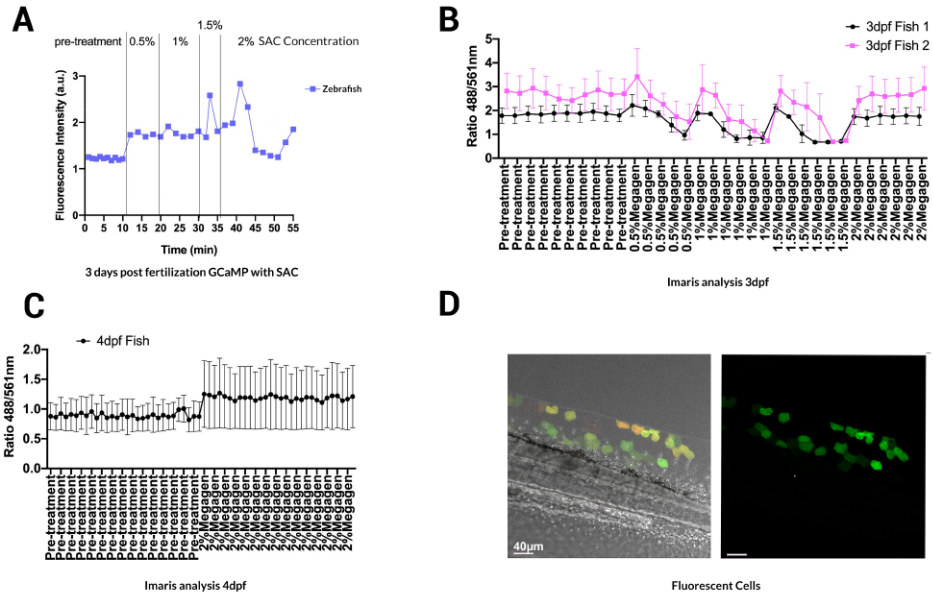


Figure 16. Effects of SAC for 12 weeks on ALP as a bone biomarker in Lab Anim Res 2011: 27(4), 301-307

SAC (Sigma Anti-bonding Calcium Carbonate)
Study with GCaMP on Zebra Fish

By Prof. Sandra Rieger, Sylvester Comprehensive Cancer Center, University of Miami, Jan. 2021



Note: in the Figure, D depicts the fluorescent cells which were analyzed. Zebrafish transient microinjections result was in a mosaic expression; therefore, only some cells expressed the GCaMP. A red fluorescent transgene was co-expressed as background control. In B, which is the data from the 3dpf fish, each in a different color. Cells were individually measured and combined as mean +/- SEM. The ration of Green (GCaMP fluorescence) was calculated at 488nm: 561nm (dsRed fluorescence). Based on the fluorescence ratios there were spikes at 3 days post fertilization(dpf) in the fish after the treatment concentration is increased. The result ware consistent with the expectation. After analyzing the data, there seemed to be a clear response at sac 0.5%, 1%, 1.5%, 2% within 2-3min.

Figure 17. Evidence of Absorption as an Ionic Calcium in Fish by Sandra Rieger, PhD, Department of Biology, Sylvester Comprehensive Cancer Center, University of Miami. Jan 2021

Observing SAC Calcium in the Cells of ZebraFish Within 2-3 Min After Taking SAC Solution

	SAC Calcium	Ordinary Calcium	Nanoparticle Calcium	Remarks
Species	SAC Calcium	Calcium carbonate, calcium phosphate, Calcium lactate, calcium chloride, Calcium oxide, egg shells, bovine bones, shells, Coral, seaweed calcium, etc.	Calcium carbonate, calcium phosphate, Calcium lactate, calcium chloride, Calcium oxide, egg shells, bovine bones, shells, Coral, seaweed calcium, etc.	
Raw material	Calcium carbonate	Most	Most	
Form	Liquid	Tablet, liquid, Powder, granule	Tablet, liquid, Powder, granule	SAC Calcium and water molecules combine with stable form
Molecular knot Combined form	Ionic antibond	Ion binding	Ion binding/ Nanoparticles	
Daily dosage	5 mg ~ 15 mg	500 mg ~ 1000 mg	500 mg ~1000 mg	
Method of administration	Dilute 5 mL of SAC Calcium solution in 500 mL of water and take it on an empty stomach	Direct administration of the liquid, tablet, granule	Direct administration of the liquid, tablet, granule	
Auxiliary ingredients	None	Added vitamin D, magnesium	Added vitamin D, magnesium	
Use	Calcium regulators Physiological ionized calcium is used for the prevention and treatment of various diseases: osteoporosis, various cancers, arthritis, diabetes, dementia, kidneys, etc.	Calcium supplements	Calcium supplements	

	SAC Calcium	Ordinary Calcium	Nanoparticle Calcium	Remarks
Absorption in the body	Through the mucous membrane, it is absorbed in the form of ionized calcium due to its self-diffusing force and electrical energy in the human body	Combined with activated vitamin D, it is absorbed in the form of protein-bound calcium	Through mucosa to the cellular membrane, or combined with active vitamin D, it is absorbed in the form of protein-bound calcium	The calcium that can function in physiological cells is ionized calcium
Absorption site	Oral mucosa, gastric mucosa	Duodenum, small intestine	Gastric mucosa, duodenum, small intestine	
Absorbed (existent) form in blood	Physiological ionized calcium	Protein bound calcium	Absorbs and exists in the form of nanoparticles, not in the form of ions	Protein and calcium can be separated from calcium only through exercise
Absorption rate	99 %	5 % ~ 15 %	40 % ~ 90 %	
Effect	The role of physiological plasma-ionized calcium: 1. Nerve transmission 2. DNA replication and information transmission 3. Information exchange between cells, activation of hormones and enzymes 4. Muscle contraction and relaxation 5. pH regulation 6. Reduce insulin resistance	No effect (only partial through exercise)	The nanoparticles pass through the cell membrane to the nucleus without resistance. The cell membrane and mitochondria are destroyed, causing inflammation and DNA mutation.	Research papers confirm side effects, toxicity, etc.

	SAC Calcium	Ordinary Calcium	Nanoparticle Calcium	Remarks
Intracellular effects	Regulate intracellular calcium concentration	N/A	Cell membrane and nucleus are present, and mitochondria are destroyed, resulting in concerns over genetic mutation	
Stem cell activation	Activation of mesenchymal stem cells, and activation of embryonic and adult stem cells	N/A	Concerns over stem cell mutation	Clinically proven
Bone mineral density (BMD) increase effects	20 % ~ 80 % increase in bone density within 3~6 months	Based on absorption rate, bone density may increase by 1 % ~ 2 %	Precipitating to the bones, bone density increases as measured by DXA measurement, but such is unrelated to the formation of bone matrix	Pass research paper certification
Effect of reducing inactivation of cancer cells and viruses	Inactivation and degradation of various cancer cells, virus inactivation effects	Excessive calcium can cause inflammation and cancer	There are concerns over nanoparticle calcium becoming a carcinogen	Mouse tumors in clinical trials
Side effects	None. However, there may be dizziness.	Calcification, constipation, and various other side effects such as inflammation and kidney stones	Inflammation, calcification, hormones, unbalanced enzyme reaction, toxicity, and safety not ensured Will be replenished due to insufficient calcium, and there will be a temporary improvement	

Table 3. Comparison of SAC Calcium and Ordinary Calcium *USA, FDA nanotechnology regulation, European Food Safety Auth. (EFSA)

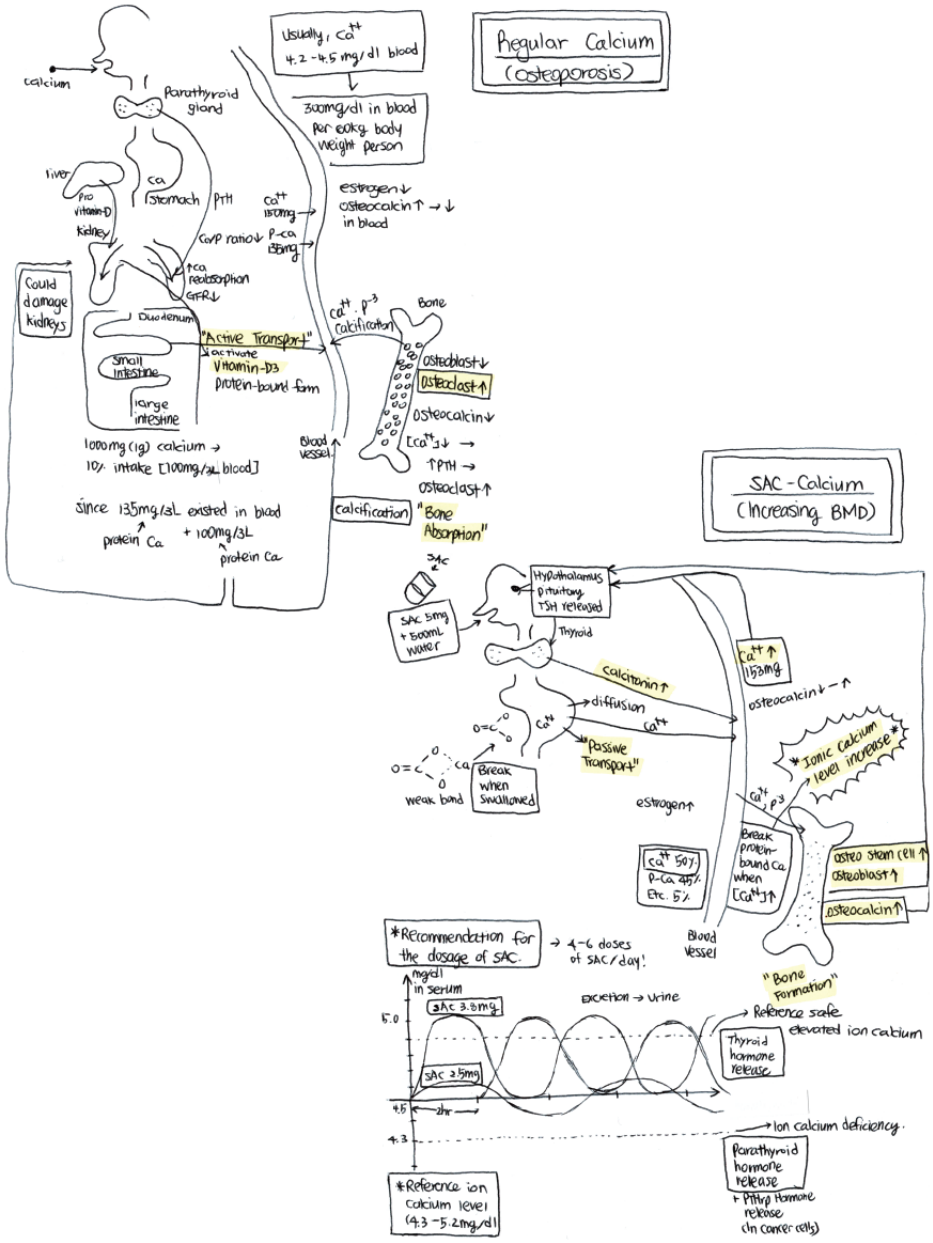


Figure 18. A Brief Depiction of Calcium Homeostasis for SAC

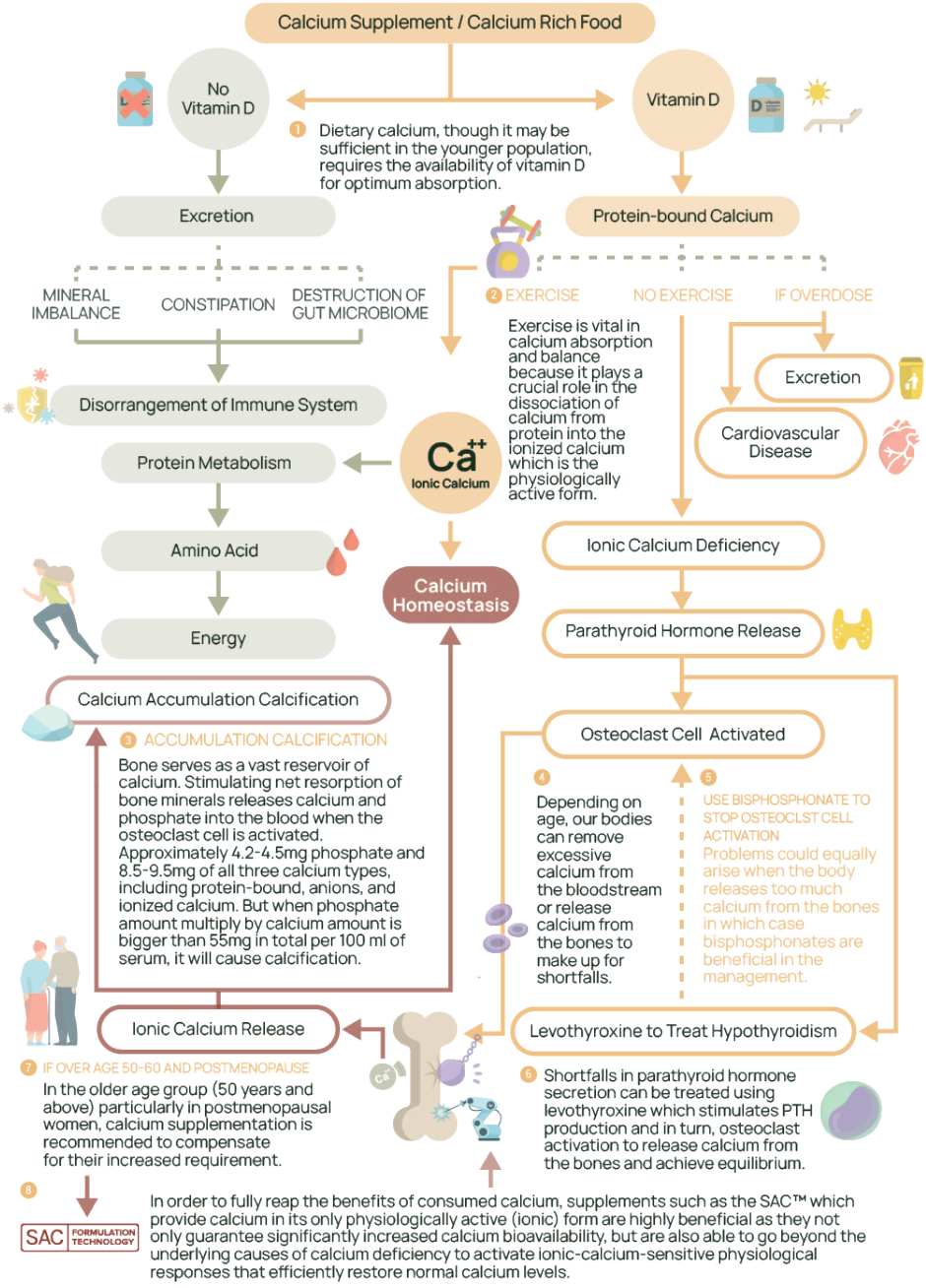


Figure 19. Calcium Homeostasis for SAC

11. How Fast Is SAC Absorbed into the Bloodstream?

"Blood takes 25 seconds to circulate the whole body, and passively diffused SAC Calcium in serum increases ionized calcium levels. Such increased levels of ionized calcium are maintained for three hours. Sensitive people can sense the effect within 30-60 seconds."

The performance of physiological calcium ions in serum can increase by 1.25×10^{19} with an amount as small as 5 mg.



Figure 20. Cardiovascular System

12. Dosage Mechanism for SAC

"The Recommended Dosage of SAC: 5-15 mg/Day/60 kg of Body Weight; Even such a small dosage can facilitate a large number of changes within your body."

Compared to the recommended daily dosage of other supplements in the market, only a small amount of SAC Calcium is prescribed for consumption along with an adequate amount of water. SAC acts as physiological ionic calcium in the blood to trigger the pituitary gland, which regulates thyroid hormone (TH) and parathyroid hormone (PTH). Moreover, as it also helps to break down protein-bound calcium in our bodies and transform them into physiologically-active ionized calcium as part of calcium homeostasis, the body's calcium signals are ultimately activated and balanced.

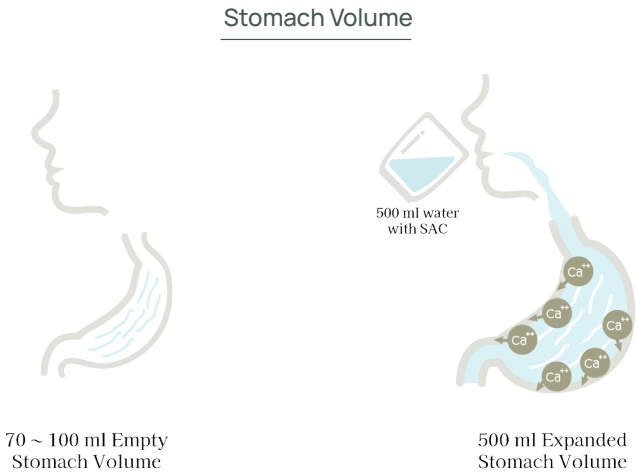


Figure 21. SAC Intake Method

Calcium in serum exists in three forms: 40 % to 45 % as protein-bound calcium, 5 % as inorganic calcium, and 45 % to 50 % as ionized calcium. In the case of a person who has a body weight of 60 kg, the level of ionic calcium in serum exists at about 175 mg Ca^{2+}/3.5 L in blood. When the amount of ionic calcium is increased up to 177-178 mg after taking 5 mg of SAC Calcium with 500 mL of water, thyroid-stimulating hormone (TSH) is released from the pituitary gland.

"The calcium absorbed with vitamin D is protein-form calcium. To convert it into physiological ionized calcium, we have to consume albumin-form protein through exercise."

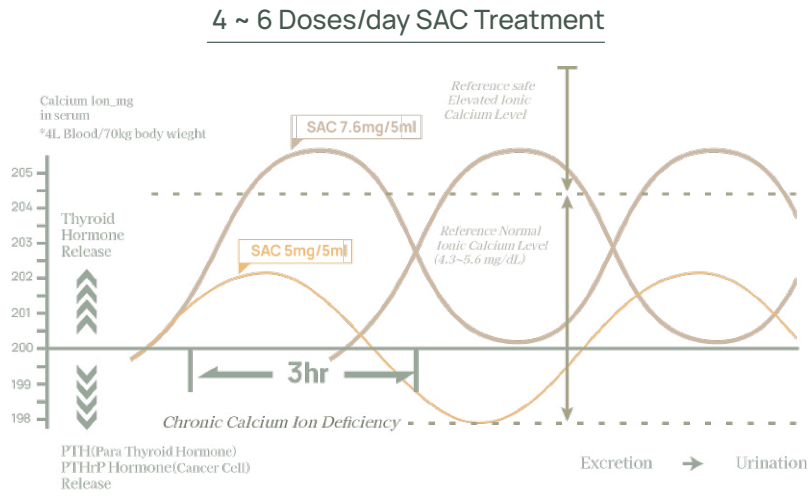


Figure 22. SAC Dosage Recommendation

As women age post-menopause, most of the increased ionized calcium in the blood is caused by the release of calcium ions from the bones due to the secretion of parathyroid hormone. The increased ionized calcium will exceed the amount required, and this excess ionized calcium is the main culprit of calcification. Conversely, SAC Calcium, calcium ions supplied from outside the body, plays an important role in the normalization of metabolism in the body. With calcium ions supplied from outside the body, after supplying cells, etc., the remaining calcium ions will be excreted from the body through urine.

12. Taking 5 mg of SAC Calcium is a Scientific Dosage

"The stable balance of calcium in intracellular and external fluids is necessary for normal cell metabolism."

All the functions of human metabolism are regulated by the autonomic, sympathetic, and parasympathetic nerves of the brain. Hormones, metabolism, etc. originate with the autonomic nerve, but if the autonomic nervous response is abnormal or irregular, it will affect all the body's metabolism functions. Which signifies that calcium metabolism is very im-

portant. It is especially important to maintain the calcium concentration in extracellular fluid during calcium metabolism. The concentration of calcium in cytoplasm is usually maintained in the range of 100 nmol/L, which is only one-tenth to 20,000th of the concentration of extracellular fluid when calcium flows out. When calcium flows in, the amount of calcium will increase by 5 to 10 times.

100 nmol/L means 4000 ng (nanogram) of calcium per l of cytoplasm. 1 ng is 0.1 billion g, and the calcium content in every 1 liter of intracellular fluid is 0.004 mg (as a reference, there are 10,000 times more calcium ions in the extracellular fluid; i,e., every 1 liter contains 40 mg). If the amount of calcium in the cell drops, the nucleus (DNA) will become abnormal, its function will decline or mutations will occur.

If 70 % of the body weight of a 70 kg adult is water, then the volume of water is about 50 l. The recommended dosage of 5 mg each time is equal to 0.1 mg per 1 liter of body weight, which is equivalent to 25 times more intake than intracellular fluid.

The reason why it is recommended to increase the calcium content to 25 times higher than that of the intracellular fluid is to rectify sodium ions. Because SAC Calcium is the absorption pathway for passive transport movement, determination was made after taking into account the amount of calcium in the extracellular and intracellular fluids.

When there is a large difference in the calcium concentration between the extracellular fluid and the cell membrane, calcium will move, and calcium will flow into the intracellular fluid quickly. This tilt of calcium concentration is maintained by the intracellular calcium reservoir, with the action of various forms of calcium pathways, including the large calcium affinity Ca^{2+}/H^{+} ATPase and the low affinity Na^{+}/Ca^{2+} exchanger.

The content of sodium is 1200 mg/kg body weight, of which 30-40 % is in bone tissue, and the remaining part is in body fluids such as cells. There are 10 % in cells and 310~340 mg/dL in serum.

Since sodium is an electrolyte, the amount in serum is more than four times that of calcium, and the content of standard heat of formation (SHF) is about three times greater.

Ca^{2+}($CaCO_3$) -288,450 cal/g mol at 25 ℃
Na^{+} (NaCl) -98,232 cal/g mol at 25 ℃
SHF is material thermodynamics energy.

As a result, the calcium of divalent cations can reach 4×3×2 = 24 times to maintain balance. Therefore, through calculation and correction, the standard recommended amount for each dose is set as 5 mg.

Therefore, for adults with a body weight of 60~70 kg, the dosage of SAC Calcium 5 mg (5 mL) + 500 mL of water per dose can be said to be a very appropriate dosage.

When supplying SAC Calcium by intravenous injection, you can refer to the following parameters: 5 %, 10 % Dextrose, 0.9 % Sodium Chloride (NS Normal Saline or NS), 0.45 % Sodium Chloride (Half Normal Saline), Lactated Ringer's (LR), Dextrose/Saline Combinations or Dextrose/LR solutions combined with glucose and normal saline or glucose and lactated Ringer's.

IV. AIC (Anti-orbital Ionic Calcium)

1. What is AIC?

AIC (Anti-orbital Ionic Calcium) is medical grade ionic calcium that is used for anti-orbital ionic calcium therapy (AICT) and pushes SAC to 99.9 % purity for extremely better absorption and achieving the highest physiological effects of SAC. AICT is currently used in thousands of medical clinics around the world.

As SAC Calcium is absorbed into the body, it is absorbed by the electric potential difference in the body (passive transport).

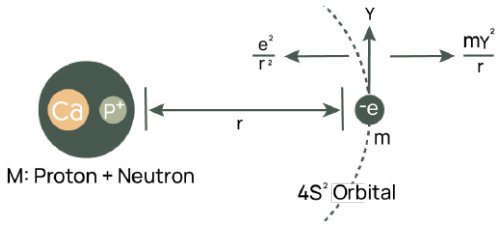


Figure 23. The Calcium Atom Energy Equation

As the force of increases, it is separated from the CO3 of AIC CaCO3. When that happens, the 4S orbital of the calcium atom disappears. The calcium atom then becomes a calcium ion. This is called anti-orbital ionic calcium.

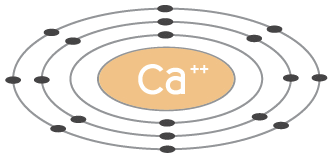


Figure 24. Calcium Ion, without Two Electrons and Orbital 4S

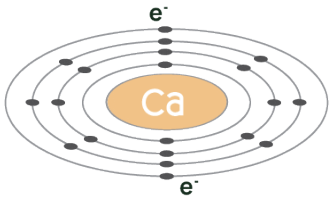


Figure 25. Calcium Atom

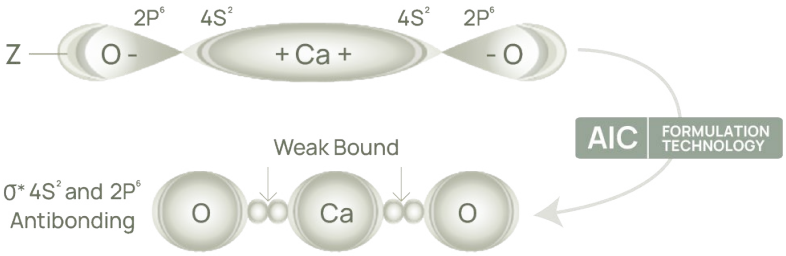


Figure 26. Orbital aAnti -bonding of Ca and O

A calcium atom has a 4s orbital in the last orbit and has 2 electrons in it. These electrons have a force of m γ 2/r, and when m γ 2/r becomes larger, the 2 electrons fall apart, and the calcium atom enters its ionic form. It becomes plasma, which becomes an indispensable element in human cell metabolism. The continuous research and development of CBHI in creating calcium ions made it possible for us to manipulate and yield an AIC CaCO3 with high electric energy; this process allows AIC CaCO3 to efficiently ionize by means of the cell voltage (electronic difference) of the human body for a certain period of time. We call this element "Anti-orbital Iconic Calcium (AIC)", and it is specifically made for medical use.

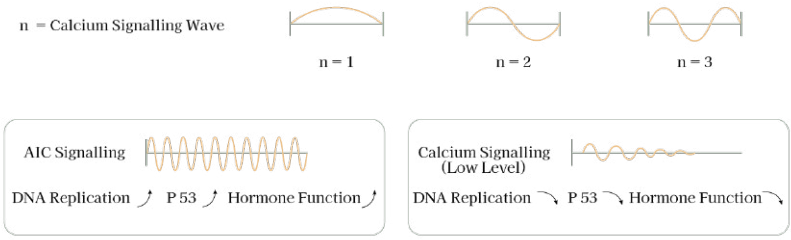


Figure 27. Calcium Signaling and Its Functions

V. SAC and Osteoporosis

"Sigma Anti-bonding Calcium Carbonate: The Innovative Approach to Treating One of the Top Ten Causes of Global Burden of Diseases: Osteoporosis"

"Sigma Anti-bonding Calcium: An innovative method to treat osteoporosis as a chronic disease that could not be cured in the past. "

"A few months ago, an 80-year-old woman who had been taking medications for osteoporosis for more than 20 years contacted me. She learned about SAC Calcium through YouTube and thought of her own osteoporosis that had not been cured through the hospital despite long-term disease treatment, wondering if SAC Calcium could really be an alternative."

"Not long ago, during the Q&A session of a seminar, a person with extensive knowledge of osteoporosis asked a question, stating that he was an active plastic surgeon and a patient suffering from osteoporosis. After a long period of time, he took the bisphosphonate series of therapeutic agents, but did not get better. Later he took SAC Calcium for three months and his bone density returned to normal."

"Ms. Mary Holt has shown a trend of recovery from multiple myeloma after taking SAC Calcium for an extended period of time, and her T-score has also significantly changed from -3.6 to -0.9, showing a trend of improvement."

1. Introduction

Osteoporosis is a skeletal system disorder associated with endangered bone strength, which is defined by low bone density and quality, consequently increasing the risk of fractures of the hip, spine, wrist, ribs, and shoulder. (Cooper, 1999) Thus it is characterized by low bone mass and

deterioration of bone tissue, which leads to decreased bone strength, increased bone fragility, and risk of fracture. (Liberman et al., 1995; Kanis, 2008; Baron & Hesse, 2012; Szulc et al., 2010) Osteoporosis has incited a critical public health issue, causing substantial morbidity and mortality.

Globally, more than 200 million women are suffering from osteoporosis, and most of these women are aged 45 years and older and have experienced postmenopause. (IOF, 2018) Moreover, nearly 8.9 million people are suffering fractures that are caused by osteoporosis. (IOF, 2018) In Europe, osteoporosis is a substantial economic burden because the disability caused by osteoporosis is greater than that produced by cancer (except lung cancer) and other chronic noncommunicable diseases such as asthma, rheumatoid arthritis, and cardiovascular diseases. (IOF, 2018)

WHO (World Health Organization) has defined osteoporosis as a BMD that lies 2.5 standard deviations or more below the average value for young healthy women. (Kanis et al., 1994) When patients are diagnosed with osteoporosis, they measure the bone mineral density (BMD) which comes with T-score value. If the result is lower than -2.5, they are diagnosed with osteoporosis, and if the T-score value is in between -2.5 and -1.0, they are diagnosed with osteopenia. If the value is greater than -1.0, they are considered normal.

T-score	BMD (g/cm^2)	Relative Loss of Bone (%)
0	1.20	0% ↓
-1.0	1.08	10% ↓
-1.5	1.02	15% ↓
-2.0	0.96	20% ↓
-2.5	0.90	25% ↓
-3.0	0.84	30% ↓
-4.0	0.72	40% ↓
-4.5	0.66	45% ↓
-5.0	0.60	50% ↓

Table 4-1. T-score and BMD Comparison Chart – Caucasian Male

T-score	BMD (g/cm^2)	Relative Loss of Bone (%)
0	1.07	0% ↓
-1.0	0.96	10% ↓
-1.5	0.91	15% ↓
-2.0	0.86	20% ↓
-2.5	0.80	25% ↓
-3.0	0.75	30% ↓
-3.5	0.69	35% ↓
-4.0	0.64	40% ↓
-4.5	0.59	45% ↓
-5.0	0.53	50% ↓

Table 4-2. T-score and BMD Comparison Chart – Caucasian Female

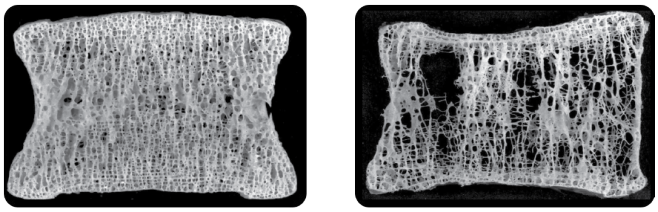
T-score	BMD (g/cm^2)	Relative Loss of Bone (%)
0	1.08	0% ↓
-1.0	0.97	10% ↓
-1.5	0.92	15% ↓
-2.0	0.86	20% ↓
-2.5	0.81	25% ↓
-3.0	0.76	30% ↓
-3.5	0.70	35% ↓
-4.0	0.65	40% ↓
-4.5	0.59	45% ↓
-5.0	0.54	50% ↓

Table 4-3. T-score and BMD Comparison Chart – Asian Male

T-score	BMD (g/cm^2)	Relative Loss of Bone (%)
0	1.05	0% ↓
-1.0	0.94	10% ↓
-1.5	0.89	15% ↓
-2.0	0.84	20% ↓
-2.5	0.78	25% ↓
-3.0	0.74	30% ↓
-3.5	0.69	35% ↓
-4.0	0.64	40% ↓
-4.5	0.58	45% ↓
-5.0	0.52	50% ↓

Table 4-4. T-score and BMD Comparison Chart – Asian Female

Although many scientists have conducted research to find a way to increase bone mineral density (BMD), current treatment options conducted in the medical setting have not yet yielded any significant increase to BMD. Bisphosphonates, SERMs, teriparatide, calcitonin and thiazide diuretics are currently prescribed to treat osteoporosis. Moreover, some physicians recommend taking calcium supplements with vitamin D, since an increase in calcium level in plasma will trigger the pituitary gland. By stimulating the thyroid gland, thyroid hormone (TH) is secreted, which initiates osteoblasts. However, the influx of calcium ions into the intracellular membrane is a pain point because the regular calcium that is sold in the market is not the physiologically-active form of calcium, as it is bound to protein. Moreover, Paziana & Paziana (2015) mentioned in their research that an excessive calcium intake could have adverse effects on the cardiovascular system because a sudden increase of calcium ions in the blood may cause calcification in blood vessels. Also, as Kim et al. (2017) discovered through their research, an excessive calcium intake can elevate the risk of metabolic syndromes for men. Therefore, due to the adverse effects of calcium intake, calcium supplements can not be the primary source for treating osteoporosis.



Normal bone matrix Bone with Osteoporosis

Figure 28. Bone Matrix Microscopic Images: Normal vs. Osteoporosis

2. The Relationship between BMD and Osteoporosis

Despite the various treatment options for osteoporosis, the prevalence of osteoporosis is increasing because the treatment options available are yet to be proven for any significant efficacy. Bisphosphonate is the most frequently prescribed medication for patients with osteoporosis. Bisphosphonate mimics the structure of pyrophosphate and activates the enzymes that are triggered by pyrophosphate. Therefore, it coordinates calcium and causes it to become accumulated in bones to increase BMD. However, it is still debatable as to whether or not BMD also improves the quality of bone. Even though bisphosphonates are the most prevalently used medication for osteoporosis, it is still unknown whether it is the best treatment option, since it only increases the density of bone and not the quality of bone.

Furthermore, IOF (2018) has identified that bisphosphonate has many adverse side effects such as hyperparathyroidism, Paget's disease of bone, osteomalacia, renal failure, and nephrolithiasis. For such reasons, whether patients with osteoporosis should continue to be prescribed bisphosphonates as their first line of medication is a subject of controversy.

SAC could be introduced as a new treatment option for osteoporosis because, as shown by Choi et al. (2011), SAC proved its medical efficacy in their research. In their study, they set out to investigate whether or not SAC increases BMD and prevents bone turnover when ovariectomized rats orally ingest 0.0012 % of SAC solution (83/μg/kg) for 12 weeks. As a result, the ovariectomized group treated with SAC had higher BMD (0.2276±0.012 mg/cm2) rates as compared to the group that was not

treated with SAC (0.1965±0.012 mg/cm2). Moreover, the ovariectomized group treated with SAC had higher 17 β -estradiol, lower osteocalcin, and type I collagen C-terminal telopeptides (CTx), which result in an increase in bone turnover.

As SAC solution is verified as a dietary supplement in Canada, the United States, Republic of Korea, Indonesia and Japan, more than 100,000 patients with osteoporosis have been using it since 2012 and showing a significant improvement in laboratory BMD tests.

In referring to one postmenopausal osteoporotic patient's data, after treatment with 5 to 15 mg of SAC orally, every day for six months, her T-score increased to -0.8 from -3.4 which was profoundly osteoporotic.

While there are no significant treatments available for patients with osteoporosis, the SAC solution can be seen as an innovative one, since it directly increases serum calcium levels as well as intracellular calcium levels, and decreases parathyroid hormone (PTH) secretion, which prevents unnecessary bone resorption. Moreover, as it has no adverse effects, due to only a small amount of calcium being ingested to trigger calcium ion concentration in the intracellular membrane, it can be an active form of treatment for osteoporosis in the future.

Group	Estradiol (pg/mL)	Osteocalcin (ng/mL)	C-terminal telopeptide (ng/mL)
Sham	4.536 ± 0.343 [a]	3.398 ± 0.294 [a]	4.751 ± 0.244 [ab]
OVX	3.920 ± 0.277 [a]	4.047 ± 0.501b [b]	5.082 ± 0.462 [b]
OVX+SAC	4.217 ± 0.247 [ab]	3.558 ± 0.154 [a]	4.360 ± 0.495 [bc]

Table 5. 17ß-Estradiol concentration and bone metabolism markers of ovariectomized rats treated with SAC for 12 weeks. Adapted from "Effects of SAC on bone turnover and calcium balance in ovariectomized rats," by S.Y. Choi et al., 2011, Laboratory Animal Research, 27(4), p. 305. Copyright 1985 by Korean Association for Laboratory Animal Science

Group	Breaking force (kg)	Ash (mg/dl)	Ca (mg/dl)	P (mg/dl)
Sham	7.113 ± 0.035[a]	428.2 ± 17.34	140.8 ± 2.48[a]	138.0 ± 3.16[a]
OVX	7.002 ± 0.106[b]	409.5 ± 8.39	136.0 ± 3.10[b]	130.3 ± 7.72[ab]
OVX+SAC	7.1220 ± 0.064[a]	419.8 ± 8.76	147.5 ± 5.33[c]	138.1 ± 5.39[a]

Table 6. Breaking force and values of ash, Ca and P of ovariectomized rats treated with SAC for 12 weeks. Adapted from "Effects of SAC on bone turnover and calcium balance in ovariectomized rats," by S.Y. Choi et al., 2011, Laboratory Animal Research, 27(4), p. 305. Copyright 1985 by Korean Association for Laboratory Animal Science

Organ (unit)	Group		
	Sham	OVX	OVX+SAC
Body weight (g)	310.84 ± 11.14	353.75 ± 10.18	371.36 ± 9.97
Uterus (g)	0.1889 ± 0.05	0.0381 ± 0.01	0.0444 ± 0.02
Vagina (g)	0.1128 ±0.03	0.0501 ± 0.02	0.0575 ± 0.02
Liver (g)	2.3435 ±0.15	2.1675 ± 0.10	2.1820 ± 0.15
Kidney			
Right (g)	0.3236 ± 0.02	0.2682 ± 0.00	0.2626 ± 0.02
Left (g)	0.3289 ± 0.03	0.2712 ± 0.01	0.2687 ± 0.01
Spleen (g)	0.1840 ± 0.02	0.1692 ± 0.02	0.1856 ± 0.02
Adrenal gland			
Right (g)	0.0127 ± 0.0024	0.0093 ± 0.0012	0.0094 ± 0.0014
Left (g)	0.0128 ± 0.0020	0.0092 ± 0.0008	0.0094 ± 0.0014
Brain	0.65550 ± 0.03	0.6044 ± 0.03	0.5526 ± 0.02
Pituitary (mg)	0.0055 ± 0.00005	0.0038 ± 0.0002	0.0042 ± 0.0010
Femur			
Right (g)	0.3296 ± 0.02	0.2960 ± 0.02	0.2902 ± 0.02
Left (g)	0.3524 ± 0.03	0.2930 ± 0.01	0.2935 ± 0.02

Table 7. The absolute organ weights of ovariectomized rats treated with SAC for 12 weeks. Adapted from "Effects of SAC on bone turnover and calcium balance in ovariectomized rats," by S.Y. Choi et al., 2011, Laboratory Animal Research, 27(4), p. 305. Copyright 1985 by Korean Association for Laboratory Animal Science

Parameters	Sham	OVX	OVX+SAC
AST (IU/L)	159.8 ± 37.8	163.0 ± 20.3	148.0 ± 30.4
ALT (IU/L)	19.2 ± 4.5	18.8 ± 4.3	22.4 ± 4.0
ALP (IU/L)	108.6 ± 29.7	112.6 ± 17.9	108.2 ± 20.7
Glucose (mg/dL)	109.8 ± 7.4	15.0 ± 17.7	112.4 ± 7.7
Triglycerides (mg/dL)	11.8 ± 6.1	19.8 ± 6.6	20.6 ± 7.7
Total Cholesterol (mg/dl)	59.6 ± 4.8	60.2 ± 24.3	73.0 ± 17.3
HDL (mg/dL)	20.8 ± 2.5	19.8 ± 6.8	23.8 ± 5.2
LDL (mg/dL)	10.2 ± 4.1	8.9 ± 5.3	8.0 ± 5.2
Total Proteins (mg/dL)	5.0 ± 0.2	4.7 ± 0.2	4.7 ± 0.2
Albumin (mg/dL)	3.0 ± 0.1	2.7 ± 0.1	2.7 ± 0.1
BUN (mg/dL)	9.7 ± 2.3	10.2 ± 2.4	9.2 ± 2.4
Calcium (mg/dL)	6.8 ± 0.3	6.7 ± 0.2	6.7 ± 0.4
Phosphorus (mg/dL)	4.4 ± 0.4	4.3 ± 0.3	4.1 ± 0.6

Table 8. Blood biochemical values of ovariectomized rats treated with SAC for 12 weeks.

3. The Classification of Osteoporosis and the Cause of Disease Development

Osteoporosis is classified into two different types: type I osteoporosis and type II osteoporosis. Type I osteoporosis is mostly seen among women who have experienced postmenopause. Type II osteoporosis is mainly observed among men with hypogonadism. According to Funaro et al. (2013), hypogonadism is "one of the main risk factors for osteopenia and osteoporosis which can be found in 8% of hypogonadal men younger than 50 years of age." (p. 19) As Chou et al., (2016) investigated in their article, "older age (> 55 years), and low ERα mRNA levels in PBLs ($\leqq$ 250.39 copies/μg DNA) were associated with an approximately 9.188-, and 31.25- fold risk of osteoporosis." (p. 124) While many scientists have assumed that osteoporosis is related to the alteration of sex hormones, the exact cause of the disease is still unknown. Moreover, a large number of scientists have aggressively researched pharmaceutical medications for osteoporosis. However, none of the pharmaceutical options have

shown significant efficacy for treating osteoporosis, and while the global population is getting older, the global economic burden of osteoporosis and osteoporotic fractures is increasing immensely.

4. Current Osteoporosis Treatments

4a. Bisphosphonate

Currently, bisphosphonate is the most frequently prescribed medication for osteoporosis. There are many different types of bisphosphonate treatments in the market such as alendronate, risedronate, ibandronate, zoledronic acid, etc. Bisphosphonate mimics pyrophosphate and acts as an antagonist, inhibiting activation of the enzyme that utilizes pyrophosphate. It also preferentially binds to calcium and promotes its storage in bones, strengthening bone health by increasing bone mineral density (BMD). According to the National Osteoporosis Foundation (2018), certain side effects have been observed in patients who were prescribed bisphosphonates. Commonly, they complained of "joint or muscle pain, nausea, difficulty swallowing, heartburn, irritation of the esophagus and gastric ulcers." (NOF, 2018) Moreover, bisphosphonates are not recommended for patients with low calcium levels, because some reports have indicated that low calcium levels may exacerbate symptoms of the disease. (NOF, 2018) Also, bisphosphonates are not recommended for patients with severe kidney disease, because it can worsen kidney calcification, which may cause nephrocalcinosis. (NOF, 2018)

Moreover, Green et al. (2010) have found out that the "prescribing of [oral] bisphosphonates over a period of about five years was associated with a doubling of the risk of esophageal cancer." (p. 545) Also, Shane (2010) claimed that long-term use of bisphosphonates can cause oversuppression of bone turnover, which can exacerbate the symptoms of osteoporosis. Moreover, Kennel & Drake (2009) have mentioned in their article, "this association was thought to be due to erosive esophagitis resulting from suboptimal administration in patients who failed to maintain an upright posture for 30 to 60 minutes after ingesting medication with a full glass of water." (p. 633). As they (Kennel & Drake, 2009) have argued in their article, the dropout rates from medication treatment is

higher among patients with osteoporosis, especially for those who are prescribed bisphosphonates. Because of its complicated administration, improper intake of the medication resulted in a decrease in the efficacy of the drug.

Through literature reviews on the efficacy of bisphosphonates for patients with osteoporosis, it has been confirmed that bisphosphonates have many adverse effects that can even exacerbate the symptoms of osteoporosis. Moreover, as the administration method for the medication is very complicated to follow, high dropout rates have been observed. Therefore, we see an urgent need in finding alternative treatment for osteoporosis with far fewer side effects and an easier administration method. For this reason, SAC would be highly recommended, as it has been proven effective in increasing BMD and decreasing bone turnover rates. Furthermore, as SAC only contains a small number of calcium carbonates that can trigger calcium homeostasis in the body, there are far fewer side effects as compared to other calcium supplements in the market.



Figure 29. Bisphosphonate Medications that are Currently Used.

Let's examine consumers' opinions on Prolia and Fosamax, which are representative osteoporosis drugs frequently prescribed by doctors in North America, through www.drugs.com.

Prolia has an average rating of 2.7 out of 10 from a total of 322 reviews for the treatment of Osteoporosis. 14% of reviewers reported a positive experience, while 79% reported a negative experience.

"Density went from -1.2 to -2.8 after taking steroids for 2 years. Prior to Prolia, started a weight bearing exercise regime to intensify bone building & planned to continue after taking the drug. I can't because Of pain! Fingers thumb & wrist, shoulders, shoulder blades, right groin, hips & pelvic area with the worst In back & neck. Muscle soreness: biceps & thighs. Jaw pain for a month. Weakness: legs, arms, &hands. Major stiffness after a short time sitting. Back cracks when I stand up, bend over or stretch. Hearing loss, fatigue & now take meds for insomnia. I was active: went to the gym, out with friends, walked 2 mi/day. No more. I awaken in the morning & can barely move. I'm depressed because of my situation. I had NONE of these Issues prior to taking this drug! Common De-denominator and cause = Prolia! This drug is poison & shame on the FDA. The makers of Prolia have put aside billions of $ for lawsuits. Hmm wonder why. I will NOT take my next injection despite what my doc advises. My whole life has been turned upside down."

One of reviews at www.drugs.com

Fosamax -Bisphosphonate has an average rating of 2.6 out of 10 from a total of 73 reviews on Drugs.com. 16% of reviewers reported a positive experience, while 83% reported a negative experience.

"Doctor prescribed Fosamax for my osteoporosis to be taken once a week. Started taking it and after a second dose my legs felt like lead and I felt irritable and depressed. Persisted for another 3 weeks until I developed a terrible skin rash and weeping sores. Saw my doctor who took me off Fosamax immediately. I will continue with Calcium and Vitamin D3 and continue to exercise and eat healthy. Side effects of Fosamax are not worth the risk."

One of reviews at www.drugs.com

4b. SERMs (Estrogen Agonist/Antagonist)

Selective estrogen receptor modulators (SERMs) are commonly prescribed for those who are suffering from postmenopausal osteoporosis, and it works by stimulating estrogenic action in bones. However, it is not recommended for women who were previously diagnosed with breast and uterine problems, because many researchers have shown the potential

side effects of SERMs for those who have such a medical history. There are many types of SERMs in the market. Raloxifene is the most commonly prescribed medication for patients with postmenopausal osteoporosis. Bazedoxifene and lasofoxifene are also commonly prescribed. While raloxifene is indicated for its efficacy in increasing lowered serum estrogen levels, its long-term use increases the risk of developing endometrial cancer by two to three times. (Maximov, Lee & Jordan, 2013) Moreover, Bazedoxifene showed an increase in the risk of experiencing venous thromboembolism. (Pickar & Komm, 2015) Since SERMs only target the repression of serum estrogen levels, they cannot control the comprehensive homeostasis of calcium in the body. Therefore, it is necessary to find an alternative approach to reviving the underlying mechanism of bone loss in postmenopausal women. Therefore, SAC can be a distinctive option in treating postmenopausal osteoporosis, while also triggering calcium homeostasis in the body.

As it facilitates balancing the serum and intracellular calcium concentration, it can increase bone mineral density as well as the quality of bones.



Figure 30. SERM Medications that are Currently Used.

4c. Teriparatide (Recombinant PTH)

Teriparatide is the recombinant form of parathyroid hormonal treatment. There are few different teriparatide medications, and one of the well-known parathyroid hormone (PTH) medications is called abaloparatide. It acts as an anabolic agent to initiate bone growth, and it is identical in form to PTH. It is used to activate more osteoblasts than osteoclasts and increases bone formation. This type of medication also manifests adverse

side effects such as limb pain, nausea, dizziness, and headache. (Rizzoli et al., 2011) Moreover, as Bazaldua & Bruder (2004) have found in their research, "[PTH] causes osteosarcomas in rats, but the relevance to humans is unknown." (p. 1983) Even though whether or not PTH may cause osteosarcoma in humans has not been extensively studied, the potential carcinogenicity of the drug may question the safety of PTH medication for some patients. Furthermore, patients who are suffering from Paget's disease of bone or open epiphysis or sudden elevation of serum alkaline phosphatase, as well as patients under radiation therapy involving bones, should not be prescribed teriparatide, since it can exacerbate related symptoms. (NOF, 2018) For this reason, it is crucial to investigate a treatment option that regulates both thyroid hormone (TH) and parathyroid hormone (PTH) to regulate bone absorption and resorption rates. Currently, PTH hormone only increases bone absorption rate by forming osteoblasts; it has no impact on bone turnover rate, which is required for improving the quality of bone. Therefore, SAC is recommended as a novel form of osteoporosis treatment, because while it triggers the circulation of ionized calcium in the body and regulates bone absorption and reabsorption rates, it can treat the fundamental cause of osteoporosis.



Figure 31. Teriparatide Medications that are Currently Used.

4d. Calcitonin

Primarily, calcitonin is produced by the thyroid gland, most often by parafollicular cells that exist in the thyroid gland. Calcitonin reduces serum calcium, and it opposes the action of PTH. It is not as commonly prescribed as other osteoporosis medications. However, some physicians prescribe calcitonin for patients with type II osteoporosis. It mainly acts

to inhibit the activity of osteoclasts. As regards minor effects, it inhibits its tubular cells in the kidney and prohibits calcium and phosphate resorption by helping urine excretion. (Potts & Jüppner, 2008) However, as Carney (1997) has identified, high calcitonin concentration in blood can increase urinary calcium and phosphate excretion, which causes hypocalcemia. Moreover, as the kidneys become resistant to calcitonin, an unregulated excretion of calcium would occur, especially in patients who are suffering from thyroid tumors, where they excrete excessive amounts of calcitonin. Also, Woo & Adachi (2001) have found that calcitonin showed "minimal changes in bone density." (p. 469) Therefore, SAC could be an alternative option for osteoporosis treatment because as it increases both BMD and quality of bone by regulating the bone turnover rate, it will be highly beneficial in the treatment of patients who are suffering from symptoms related to osteoporosis.



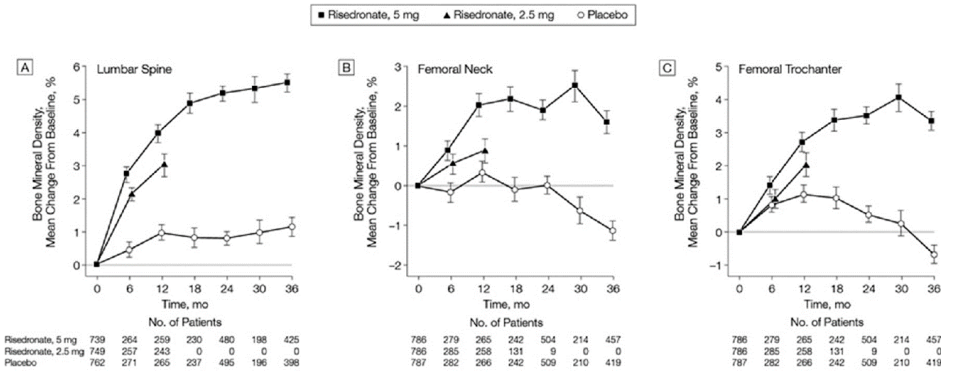
Figure 32. Calcitonin Medications that are Currently Used



Figure 33. RANKL Inhibitors that are Currently Used. Such work by preventing the development of osteoclasts, which are cells that break down bone.

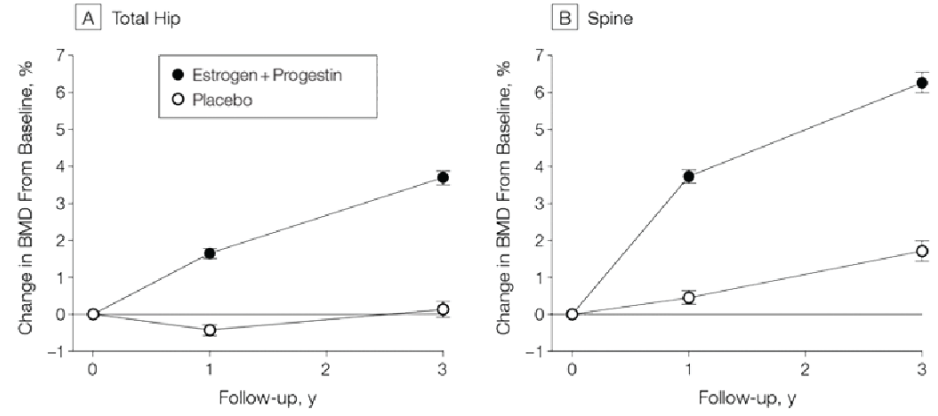
4e. Bone density increase effects in bisphosphonate therapy for osteoporosis

In terms of the bone density-increasing effect of the bisphosphonate series of osteoporosis treatment agents, the representative effects of taking risedronate sodium and alendronate sodium are: an increase of approximately 1 to 2 % per year.



Error bars represent SEM. Measurements at 6, 12, 18, and 30 months were performed at a subset of study centers. In A, $P<.05$ vs baseline for all time points and groups except the placebo group at 6 months; $P<.05$ vs placebo for all time points in the 2.5-mg and 5-mg risedronate groups. In B, $P<.05$ vs baseline for all time points and groups except the placebo group between 0 and 24 months; $P<.05$ vs placebo for all time points in the 5-mg risedronate group and at 6 months for the 2.5-mg group. In C, $P<.05$ vs baseline for all time points and groups except the placebo group at 30 months; $P<.05$ vs placebo for all time points in the 5-mg rised-

Figure 34. Risedronate: 1-2 % per year; increase in bone density in the spine and thigh.



BMD indicates bone mineral density. Error bars indicate SEs.

Figure 35. Estrogen agonists: 1-2 % per year; increase in bone density in the hip and spine.

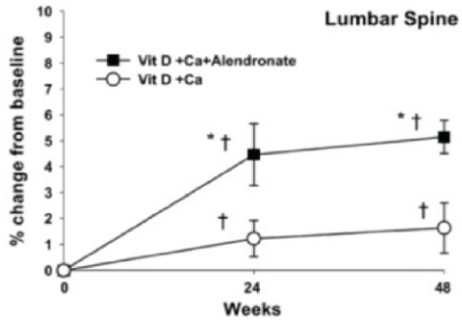


Figure 36. Changes in bone mineral density (± SEM) in the lumbar spine (primary end point of the trial) after 48 weeks of alendronate, vitamin D (Vit D), and calcium (Ca) versus vitamin D and calcium alone. *Significant change from baseline ($P < 0.05$). *Significant difference between arms ($P < 0.05$)

4f. Side effects of taking calcium supplements and high dose calcium supplements (kidney failure)

In order to maintain bone health, many people will have calcium supplements on hand at home, school and work for convenience. In addition, we can easily purchase high-calcium foods. Even if you consume a large amount of calcium every day by means of a diet consisting of milk, cheese, and anchovies, or consume calcium supplements and vitamin D that are recognized to be high in absorption, it is difficult to directly increase bone density. In addition, an overdose of calcium may be harmful, so special attention is needed.

As mentioned earlier, American cardiologist Dr. Thomas Raby stated in his book "Death By Calcium" that excess calcium in cells has great side effects on the human body, so strongly recommended not taking calcium supplements. When taking 500 mg of calcium daily, the probability of suffering from cardiomyopathy and stroke increases by 30 % and 20 %, respectively. More than one-third of the arteries of American adults over 45 years old have calcification. Cell inflammation becomes the cause of chronic diseases and cancer. In addition, MRI devices have detected that malignant calcification can develop in 95 % of the prostate. It is noted that the thyroid is also exposed to the risk of calcification, which induces inflammation until it progresses to cancer. The National Health and Nutrition Examination Survey (NHANES) in the United States conducted a survey on the eye disorder macular degeneration in the elderly, and

found that those who took 800 mg of calcium supplements a day had 85 % higher rates of macular diagnosis than those who did not.



Figure 37. Macular Degeneration of the Eye

In addition, if there is a calcium imbalance in the kidneys due to excessive calcium, a person will be exposed to threats such as calcification, cardioplegia, arteriosclerosis, stroke, conjunctivitis, macular degeneration, and glaucoma (Heine, Nangaku & Fliser, 2012).

4g. Calcium and the kidneys

The kidneys have the function of filtering waste in the body and excreting it out of the body through urine, as well as the function of re-absorbing the components required by the human body. The kidney is one of the most delicately designed organs in the human body. One million microvessels with a size of 0.1 mm are interwoven with silk spheres to recover the required components and discharge waste through urine. Most people say that "it is difficult for the kidney to regenerate once damaged." In fact, once renal function declines, it will proceed down a path of deterioration, where most patients with kidney dysfunction will eventually have to undergo dialysis or kidney transplantation. Calcium ions play an important role in maintaining kidney function in the silk spheres and thin tubes of the kidneys (Gallant & Spiegel, 2017). SAC Calcium has excellent effects in treating nephritis and restoring renal function.

"In November 2013, Molly Holt had a renal GFR (Glomerular Filtration Rate) of about 10 % before taking SAC Calcium. However, a review in July 2017 found a maintained GFR value of more than 60%. Various blood indicators reflected overall good results."

"A man living in Vancouver (72 years old, 2016), due to urinary reflux of the bladder, had a renal function that was greatly impaired and a GFR that dropped to 9%, so the hospital diagnosed that he needed dialysis treatment. However, he took SAC Calcium and his GFR rose to 23 % after a month."

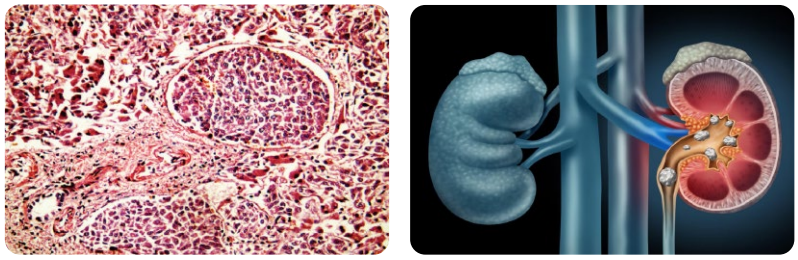


Figure 38. Microscopic Observation of Kidney Tissue (black spots are inflammatory cells)

4h. Calcification

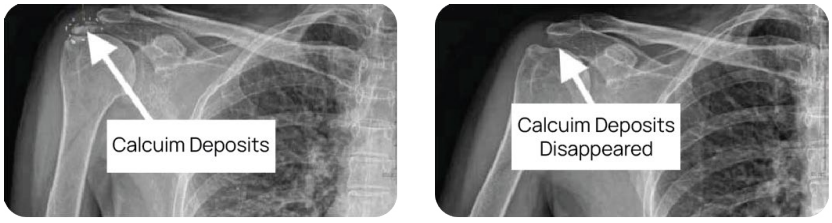


Figure 39. Dosage - SAC 2 x 250 ml/day for the first 2 months and then only 1 x 250 ml/day thereafter. Able to walk normally again

An overdose of calcium is the cause of calcification in human tissues (Singh & Ashraf, 2012). So does an overdose alone cause calcification? Obviously not. In addition, when calcium metabolism is not balanced, or calcium is lost from the bones, it can also cause calcification (Wang et al., 2010). When calcium (Ca)×(P) in the blood or in cells is greater than or equal to 55~60 mg^2/dl^2, calcification can occur (Karwowski et al., 2012).

Of course, the variance in the individual vascular environment is also very important. Even if it exceeds 55-60 mg2/dl2, some people will not develop calcification, and some people will experience calcification if

it is less than this value (Karwowski et al., 2012). Calcification occurs everywhere in the human body, from the heart, kidneys, blood vessels, muscles, etc. to various places around the eyes. Calcification usually starts from the mitochondria or the plasma reticulum membrane of the mother cell, while mitochondria develop and begin to calcify within the entire cell in daughter cells (Carafoli, 2010).

Calcification from the outer membrane of the cell, due to hemorrhage or cell destruction, turns to the outer membrane to develop and develops into calcareous dry inflammation, apatite degenerative osteoarthritis, bamboo arteriosclerosis, heart valve calcification, arteriosclerosis, and others calcification diseases, etc. (Lerman, Prasad & Alotti, 2015). Calcification is the crystallization of hydroxyapatite (hydroxylapatite). Generally, calcification begins due to the interaction of hyper-phosphatase and calcium-bound phospholipids, which is determined by the concentration of calcium and phosphorus (Boskey, 1978). Calcification can cause intracellular inflammation, become the cause of degenerative diseases, and even become the beginning of cancer (Hristva et al., 2011).

4i. Does an overdose of SAC Calcium also cause calcification or dissolving it?

In terms of the possibility of an overdose of SAC Calcium that forms calcification, not only is it impossible for SAC Calcium itself to calcify, but it will actually dissolve calcified substances. SAC Calcium is not obtained from the bones, but from outside the body to increase calcium ions. It increases the pH of the blood, decreases the value of calcium × phosphorus = calcification 55 - 60 mg^2/dl^2, and plays the role of dissolving calcification.

A single dose of SAC Calcium is 5 mg; assuming 100 % is absorbed into the blood, ionized calcium will increase by 5 mg.

Although blood volume varies according to body weight, it is generally from 3.5 L to 5 L. This can be understood as an increase of 0.125 mg of calcium per 100 ml of plasma. On average, human plasma calcium is about 9.5 mg/dL and phosphorus is 4.5 mg/dL. The effect of phosphorus during calcification is almost twice that of calcium. As a result, taking SAC Calcium will dissolve the calcification. Through clinical experience,

we have observed many cases where calcifications in breasts, shoulder muscles, and the heart were dissolved by means of SAC Calcium.

Although many people worry that taking SAC Calcium will result in excessive calcium ions in the blood, due to the homeostasis of calcium, only a certain amount of necessary calcium ions will remain in the cells, and the excessively accumulated calcium ions will quickly be discharged through urine. Therefore, even when adults with no special medical knowledge take SAC Calcium, it is certain that excessive calcium ions that have accumulated in the body are excreted through urine within 30-40 minutes after consumption of SAC.

When ionized calcium in the blood increases after taking SAC Calcium, in addition to filling the cells, the remaining ionized calcium will be quickly excreted from the body through urine. When healthy people take SAC Calcium, after ionized calcium in the blood increases, the remaining ionized calcium will be excreted through urine.

4j. Calcium channel blockers

"Some doctors believe that inflammation and cell necrosis in cells are caused by excessive calcium influx in the cells. Therefore, calcium channel blockers are recommended to patients. Could calcium channel blockers become a fundamental solution?"

As our age increases, the amount of ionized calcium in the blood decreases, and parathyroid hormone is secreted to obtain a calcium supply from the bones. As hormone sensitivity decreases, the calcium flowing out of the bones will exceed the required amount. As calcium flows out, phosphorus also flows out.

Usually when the plasma content is greater than calcium × phosphorus = 55 mg2/dl2, it will cause intracellular inflammation, DNA mutation, calcification, and lead to cell destruction and mutation during cell division. During calcification, the intracellular calcium concentration occasionally drops dramatically.

Although using a calcium channel blocker to block the influx of calcium seems to be a convincing option, and metaphorically speaking there is still the view that only 'vascular trees' can be seen. If we do not

completely block the flow of calcium from the bones, but only focus on blocking the flow into the endoplasmic reticulum, can the problem be solved? The only way to prevent the outflow of calcium from the bones is to inactivate osteoclasts by injecting bisphosphonates, but this will cause other problems. To solve these new problems, other new measures must be developed.

The best way to maintain the amount of ionized calcium in the blood is to supplement it from outside the body. However, calcium supplemented from outside the body will be absorbed by vitamin D and absorbed into the blood in the form of protein-bound calcium. For this calcium to become ionized calcium, the protein must be removed. The only way to do this is through exercise, but ionized calcium must be reused during exercise. So there is no way for the elderly to increase ionized calcium in the blood.

Therefore, as SAC Calcium can be absorbed in the form of ionized calcium in the blood without vitamin D, we can conclude that it is the only viable substance. After SAC Calcium enters the blood, it will first block the flow of calcium and phosphorus from the bones. By regulating the calcium channels, the calcium concentration in the cells tends to be normalized. Even Parkinson's disease can be treated, which is powerful evidence.

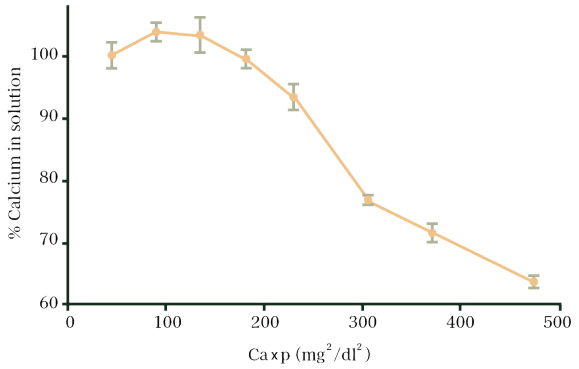


Figure 40. Graph of Calcium Phosphate Precipitation in Human Plasma. The plasma was anticoagulant lated with 15 U/ml heparin and cultured at 37 °C and 5 % CO2. After adding the 45Ca tracer, CaCl2 and buffered sodium phosphate were sequentially added starting from the 1M solution. Samples were taken 15 minutes after each feeding, and 16 g samples were centrifuged for 3 minutes, which is the average ±s.e of three iterations. When the calcium content is low, even if the Ca x P is high, there is less calcification in the liquid

VI. SAC and BMD Clinical Data

"For decades, to find a suitable therapeutic agent for osteoporosis, many researchers have made arduous and unremitting efforts. But so far, they have not developed a therapeutic agent that can significantly increase and restore reduced bone density. During this process, the research group developed SAC Calcium, a novel calcium preparation, which proved effective in increasing bone density through animal experiments."

We used BeamMed™ MiniOmni™, conducted testing using the distal radius test method, and to avoid experimental bias, we performed the test three times. Moreover, we also used an ultrasound BMD densitometer to get more accurate results. Women who are 55 and older who experienced postmenopause have the highest risk of experiencing type I osteoporosis. We have investigated the BMD data for women aged 55 years and older after treating them with Sigma Anti-bonding Molecule Calcium Carbonate (SAC) for several months. The data below depict the difference in BMD after treatment with SAC.

1. Introduction

In order to make it easier to measure the bone density before and after taking SAC Calcium, CBHI proposed the use of the BeamMed™ MiniOmni™ ultrasonic bone density meter to measure the radius of the wrist bone, thus avoiding any bias of the experimenter. The average value of the ultrasound and reflection velocity is calculated, repeated 3~5 times, and the DEXA measurement value is used to correct and for conversion to bone density (BMD) (g/cm^2), and the bone density of 35-40 years old is used, which is the optimal age range for bone health over a lifespan. Then the current T-score is obtained as a benchmark. The results of the test were performed on post-menopausal women over 55 years of age. Most of the women suffer from type I osteoporosis, bone deficiency, or are at risk

of suffering from these symptoms. After that, they were given SAC Calcium every day. Three months thereafter, the bone density was measured. The results indicate that the bone density index was greatly improved.



Figure 41. Ultrasonic bone densitometer

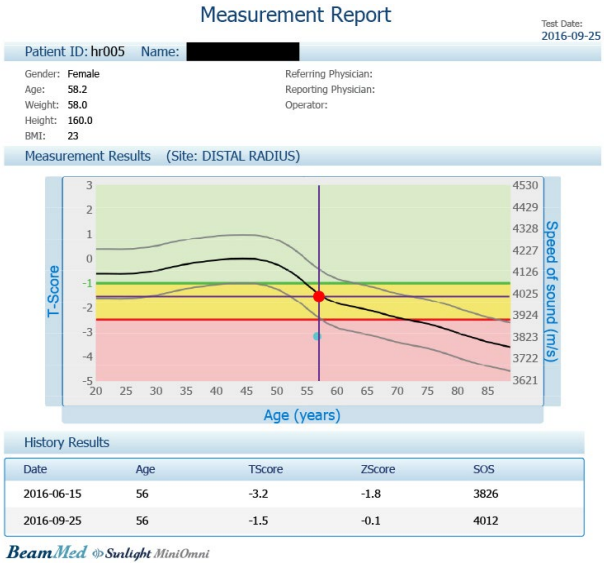


Figure 42. BMD Change in an Asian Woman, Aged 56 Years Old After Treatment with SAC for 3 Months

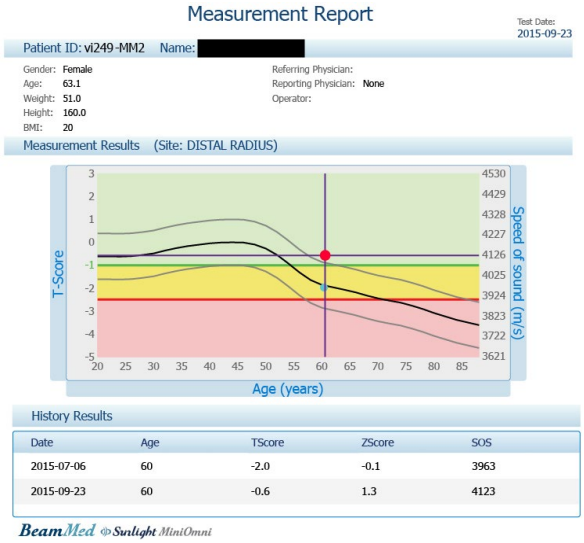


Figure 43. BMD Change in an Asian Woman, Aged 60 Years Old After Treatment with SAC for 3 Months

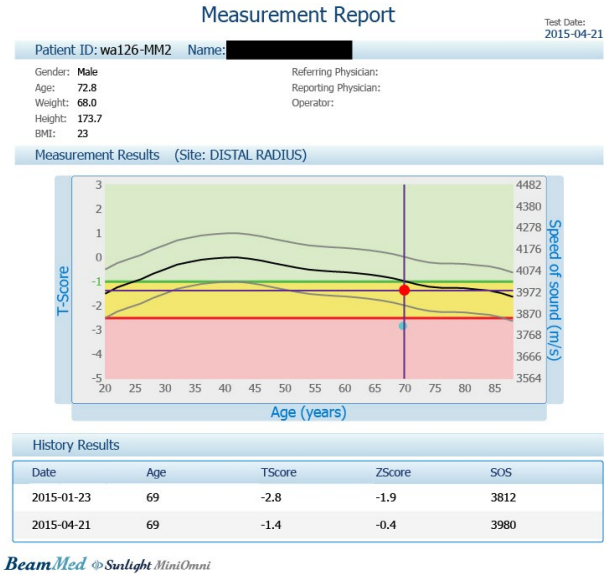


Figure 44. BMD Change in an Asian Woman, Aged 69 Years Old After Treatment with SAC for 3 Months

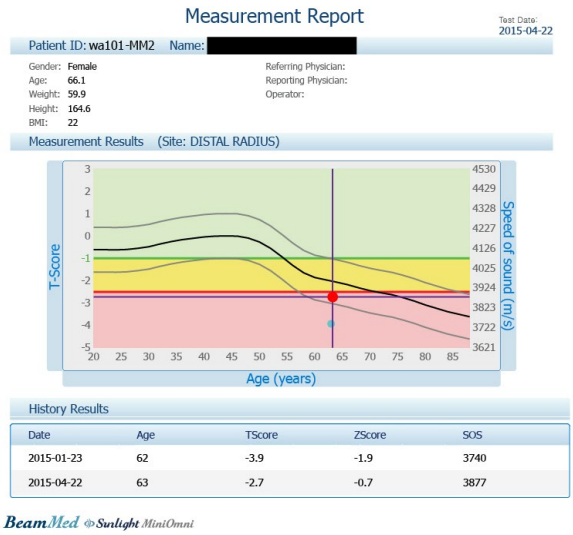


Figure 45. BMD Change in an Asian Woman, Aged 62 Years Old After Treatment with SAC for 3 Months

2. The Effect of a Small Amount of SAC Calcium on Increasing Bone Mineral Density

Some patients discuss with their attending doctor whether they need to take SAC Calcium. Most doctors say that the calcium content of SAC Calcium is too low to provide sufficient calcium nutrition.

In early 2012, for clinical trials, we sent SAC Calcium to Texas, USA. We were contacted by the FDA shortly after passing US customs and delivering the goods to the destination.
They said that because the amount of calcium is too small, it cannot be certified as a calcium supplement.

Compared with general calcium supplements, the calcium content of SAC Calcium is only about 1/100~1/200. In addition, it is only 1/50 of the calcium supplement specifications published by the FDA, which does not meet their regulations.

How is it possible to increase BMD (bone mineral density) in a short period of time (3-6 months) with a small amount of calcium (10~15 mg/

day) without side effects?

The amount of calcium in a healthy body is about 1-1.3 kg based on the weight of men and women. Bone is composed of calcium, phosphorus, magnesium, collagen, water, etc., accounting for about 12-15 % of our body weight.

A young male has about 1.3 kg of calcium, but as he ages, bone loss occurs, up to about 30 %, and calcium quantities will decrease to about 910 g. As that happens, the T-score drops to about -3, and it is easy to develop bone porosity.

1) Mrs. A's T-score value is -3.2, which indicates that she's a patient with osteoporosis. Therefore, it's recommended that she take 5 ml of SAC Calcium solution with 500 ml water 2 times every day, and the T-score value will return to -0.8 after 90 days of continuous use. In other words, although the amount of calcium in the body has increased from 884 g to 1196 g, the total amount of calcium that A actually took was only about 900 mg, that is, only about 0.3% of the increased calcium amount of 312 g, but bone density has increased by 37.5 %, and the amount of calcium in the body increased by 35%. This can be explained as SAC Calcium promotes calcium homeostasis and plays a role in regulating calcium ion secretion.

2) Mrs. B's T-score value is about -1.7, which is a value in the range of risk for osteoporosis, so it's recommended that she take SAC Calcium. It's also recommended that she take 10 ml a day, and to take it regularly for three months. As a result, the T-score value improved, rising to about -1.2. Assuming that the original calcium content was about 1000 g, the calcium content in the body decreased to 830 g after bone density decreased, but after taking SAC Calcium, there's a confirmed increase to 880 g. But the total amount of SAC Calcium taken by B was only 360 mg, how is it possible that the amount of calcium increased by about 50 g? It can only be explained that SAC Calcium acts as a calcium ion promoter. It's astonishing, isn't it? After only taking 360 mg of SAC Calcium, the bone density increased by 24.4 % and the calcium in the body increased by 6%, which is surprising.

VII. SAC in Mitochondria and Stem Cells

"Sigma Anti-bonding Calcium Carbonate: Helps to Regenerate Mitochondrial Function and Initiate Mesenchymal Stem Cells Activity Which is the Key to Treating Chronic Neurodegenerative Diseases, Including All Cancers."

Do you know what percentage of your body is occupied by mitochondria?

Mitochondria shares 20-23 % of your body weight.

1. Introduction

Within the past ten years, many significant pieces of research have been conducted to investigate the relationship between mitochondrial dysfunction and the development of chronic neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and Alzheimer's disease (AD). Recently, there were also notable findings that indicated an increased influx of calcium ion causes the excitotoxicity of mitochondria, which results in chronic neurodegenerative diseases.

Therefore, it is critical to balance out the ionized calcium concentration in the cytosol and mitochondrial matrix to prevent an over-influx in calcium concentration in the mitochondrial matrix, which results in mitochondrial dysfunction. Moreover, mitochondrial dysfunction increases the production of reactive oxygen species (ROS), which affects the alteration of RNA synthesis, which mutates mitochondrial proteins such as SOD1, FUS, and TDP-43. The misfolding of the proteins would alter permeability for ionized calcium. Therefore, as the calcium buffer system collapses, calcium excitotoxicity would occur, which is the primary cause of the development of chronic neurodegenerative diseases. Thus, it is crucial to regulate the ionized calcium concentration in the cytosol and mitochondrial matrix.

To control calcium homeostasis, regulating thyroid hormone (TH) and parathyroid hormone (PTH) is also essential to solving an imbal-

ance in calcium homeostasis. While mitochondrial dysfunction results in chronic neurodegenerative diseases, due to the alteration of RNA, DNA and protein synthesis, it is also crucial to regenerate mitochondrial function by triggering depolarization of the mitochondrial intracellular membrane. As Duchen (2000) has insisted in their article, calcium uptake depends on mitochondrial membrane potential, which is followed by transient mitochondrial depolarization. Therefore, calcium signaling should be switched on by exposing the small amount of calcium in cytosol that activates the action potential in the mitochondrial matrix. Impaired mitochondria can be restored through the regeneration of calcium depolarization by increasing the permeability of calcium in the mitochondrial matrix. As mitochondrial function is regenerated, more ATP is produced, which may initiate mesenchymal stem cells (MSCs); and this can be the key to treating chronic neurodegenerative diseases. For instance, as ALS occurs due to the dysfunction of mitochondria in muscle cells, if the mitochondria are regenerated and able to produce sufficient amounts of ATP, such can activate MSCs, which are located in muscle tissue. Therefore, as the muscle cells are regenerated, the symptoms of ALS can be treated. Since the problem is how to regulate calcium homeostasis in the body, we can assert that SAC can act as a trigger to initiate calcium homeostasis. As Choi et al. (2011) have identified, SAC has shown its efficacy in regulating calcium homeostasis by exhibiting a change in bone mineral density (BMD) in mice; which is indicated by the secretion of TH and PTH hormones.

2. Mitochondrial Dysfunction and the Development of Chronic Neurodegenerative Diseases

Damiano et al. (2006) have found in their research that poor calcium uptake mutates SOD1, which ultimately causes defection in spinal cord and brain tissue. Moreover, Nguyen et al. (2009) investigated that mitochondrial inner membrane depolarization depends on "calcium influx into motor terminals." (p. 2009) By analyzing their experimental results, it seems clear that mitochondrial defects can mutate protein uniporters. Mutated protein uniporters, such as SOD1, can cause the alteration of calcium uptake into the mitochondrial matrix. Elevated intracellular calcium con-

centration contributes to excitotoxicity in the mitochondrial membrane (Kawamata & Manfredi, 2010) and it ultimately leads to mitochondrial dysfunction. Moreover, mitochondrial dysfunction elevates ROS production (Wei et al., 1998), which can cause damage to lipids, DNA, and proteins; this ultimately causes aging-related chronic degenerative diseases. (Lin & Beal, 2006) Therefore, it is crucial to regenerate mitochondrial dysfunction.

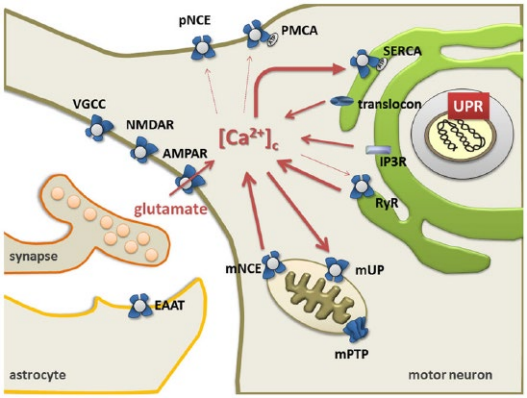


Figure 46. Calcium dysregulation, ER stress and mitochondrial impairment are major components of excitotoxicity in motor neurons. Mitochondrial dysfunction causes activation of proteolytic- and ROS-generating enzyme systems. Mutant SOD1 forms insoluble aggregates in mitochondria at the surface of the outer membrane. Motor neurons might also undergo transcriptional dysregulation and abnormal RNA processing which, together with depleted ER Ca2+ levels and overproduction of ROS, contribute to aberrant protein folding. Aberrant proteins form aggregates lead to ER stress and ultimately to activating apoptotic pathways, especially when the unfolded protein response is exhausted. Impaired axonal transport may also contribute to an energy deficit in the distal axon and the dying-back axonopathy that is observed in ALS. Adapted from "The ER Mitochondria Calcium Cycle and ER Stress Response as Therapeutic Targets in Amyotrophic Lateral Sclerosis," by V. Tadic et al., 2014, Frontiers in Cellular Neuroscience, 8 (147), p. 10. Copyright 2014 by PMC

Amyotrophic lateral sclerosis (ALS) is a well-known chronic neurodegenerative disease that is caused by calcium excitotoxicity in the intracellular mitochondrial matrix. Kawamata & Manfredi (2010) mentioned that the relationship between calcium dysregulation, mitochondria dysfunction, and neuronal cell death is the key to understanding ALS. They also stated that mutation of SOD1 is the key to explaining the occurrence of ALS. Initially, SOD1 works as an antioxidant to remove superoxides, which is produced in the respiratory system. Mutation of SOD1 results in mitochondrial dysfunction, which is impaired calcium handling. (Kawamata & Manfredi, 2010) It leads to disturbance in the homeostasis of cal-

cium ions. An imbalance of calcium ion levels results in neuronal cell death ,which occurs in ALS.

Moreover, Lüdtemann & Abramov (2018) have mentioned that calcium dysregulation is significantly related to Parkinson's disease. This research shows that mitochondrial calcium ion overload and dysfunction is related to PD-risk genes. One of the PD-risk genes is α -synuclein. (Activation of alpha-synuclein induced calcium ion dysregulation and oxidative stress.)

Another PD-risk gene is PTEN-induced putative kinase 1 (PINK-1). "Ca^{2+} extrusion in PINK-1 deficient neurons were severely inhibited, resulting in mitochondrial Ca^{2+} overload, increased reactive oxygen level species production, PTP opening, and ultimately neuronal cell death." (Lüdtemann & Abramov, 2018, p. 88) The mechanism of PD-risk genes hinders the efflux of calcium ions and ultimately leads to cell death, which occurs in Parkinson's disease.

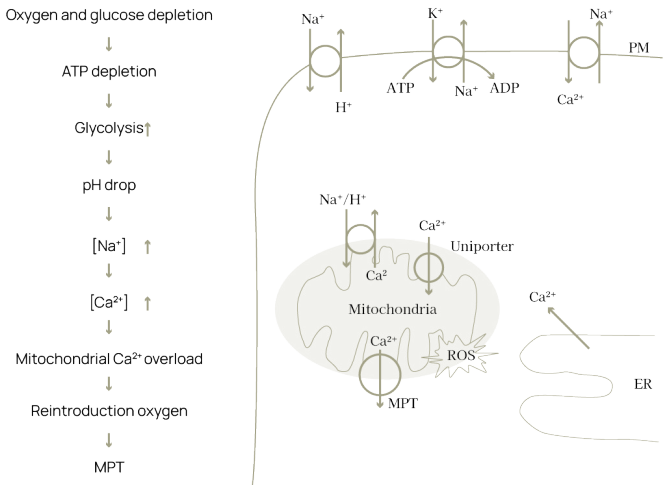


Figure 47. Mechanisms of mitochondrial calcium overload and subsequent mitochondrial permeability transition. Depletion of oxygen and glucose during ischemia induces the shutdown of oxidative phosphorylation and results in ATP depletion. The consequent increase in anaerobic glycolysis lowers pH. To counteract this pH drop, the cell activates the Na+/H+-antiporter, causing an increase in Na+. The excess Na+ cannot be pumped out via the Na+/K+-ATPase because of reduced ATP availability. So, the cell becomes loaded with Ca2+ due to reversal of the Na+/Ca2+-antiporter. In addition, cytosolic Ca2+ can also be elevated by Ca2+ influx from the ER. Calcium entering the mitochondria via the Ca2+-uniporter cannot be pumped out by the mitochondrial Na+/Ca2+-antiporter due to saturation, and the result is mitochondrial calcium overload. Reperfusion re-introduces oxygen, the damaged mitochondria produce a burst of ROS, and intracellular pH is restored. These changes, in combination with the calcium overload and other factors, induce MPTP. Adapted from "Molecular Mechanisms and Pathophysiology of Necrotic Cell Death," by N. Vanlangenakker et al., 2008, Current Molecular Medicine. 8(3), p. 209. Copyright 2008 by Bentham Science Publishers

3. SAC and Calcium Homeostasis

SAC plays a critical role when ingested into the circulatory system, because it acts as a hormone trigger, which initiates calcium homeostasis. Calcium homeostasis is essential in triggering calcium influx in the intracellular mitochondrial matrix. As Kawamata & Manfredi (2010) have insisted, calcium homeostasis maintenance is required to make calcium influx dynamic. In other words, to initiate mitochondrial function and regulate calcium influx and efflux in the mitochondrial matrix, controlling the secretion of thyroid hormone (TH) and parathyroid hormone (PTH) is essential. Moreover, Loskutova et al. (2009), have found that bone mineral density (BMD) is linked to the "bone loss in early Alzheimer's disease." (p. 1) According to their research, in the early stages of Alzheimer's disease, patients have exhibited significant bone loss. (Loskutova et al., 2009) Moreover, brain atrophy and memory loss have frequently been observed among patients who experienced early symptoms of Alzheimer's disease. (Loskutova et al., 2009)

Based on their research, we suppose that if SAC can increase BMD rates, it would prove that SAC acts as a trigger to initiate calcium homeostasis. Also, if calcium homeostasis is controlled, we can also regulate intracellular calcium influx and efflux; which means that we can eventually regenerate mitochondria. While mitochondrial modification is essential to stem cell initiation and restoring motor neurons, SAC can be the key to treating chronic neurodegenerative diseases.

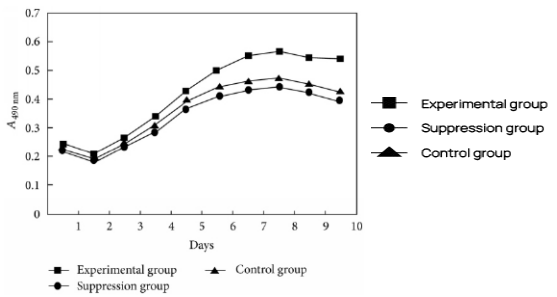


Figure 48. Growth Curve of BMSCs in Experimental, Suppression, and Control Groups ($\Delta < 0.001$ between the experimental group and the suppression group and control group; $* > 0.001$ between the suppression group and control group). Adapted from "Effects of Parathyroid Hormone on Calcium Ions in Rat Bone Marrow Mesenchymal Stem Cells," by Y. Chen et al., 2014, BioMed Research International. p. 3, Copyright 2014 by Hindawi

Group	n	Calcium Ion Intensity	
		7d	14d
Experimental Group	6	7.91 ± 2.34*	5.15 ± 1.58**
Control Group	6	5.37 ± 2.55**	2.71 ± 0.89*

** P < 0.001 between two samplings, or between groups.
* P > 0.001 between the experimental group on the 14th day and the control group on the 7th day.

Table 9. Calcium Ion Fluorescence Intensity of BMSCs in the Experimental and Control Groups. Adapted from "Effects of Parathyroid Hormone on Calcium Ions in Rat Bone Marrow Mesenchymal Stem Cells," by Y. Chen et al., 2014, BioMed Research International. p. 4, Copyright 2014 by Hindawi.

As Choi et al. (2011) found in their research, SAC plays a crucial role in elevating BMD, as well as enhancing bone quality. They orally administered a 0.0012 % SAC solution to ovariectomized rats for 12 weeks. They also examined changes in osteocalcin, type I collagen C-terminal telopeptides (CTx), 17 β-estradiol, breaking force, BMD, bone ash, and calcium and phosphorus in femurs. They compared the control (sham) with two different groups: ovariectomized group (OVX) and SAC-treatment group (OVX+SAC). The experiment results show that SAC has significant efficacy in elevating 17 β-estradiol and osteocalcin, as well as decreasing CTx rate. Moreover, it has been demonstrated that SAC has considerable IX effectiveness in increasing breaking force, BMD, bone ash, as well as calcium and phosphorus in femurs. As renal excretion also occurs when calcium homeostasis is regulated, they compared the mass of rats' kidneys. The results showed a significant decrease in renal mass. Therefore, we are able to conclude that SAC acts as a hormone trigger in the body to initiate calcium homeostasis.

4. SAC and Calcium Signaling for Mitochondrial Regeneration and Mesenchymal Stem Cell Initiation

Calcium signaling has a profound effect on initiating mesenchymal stem cells (MSCs), because as calcium ions influx and efflux through the Ca^{2+} channel, action potential is activated, which gives initiating signals to MSC. According to Zhang & Morad (2016), "the contribution of mitochondrial Ca^{2+} signaling to the spontaneous beating activity of human-in-

duced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) has been recently examined." (p. 102)

Their research yielded that mitochondrial calcium signaling is essential to inducing the activity of MSCs; which means that the imbalance of intracellular and extracellular calcium ions can cause mitochondria-related chronic degenerative diseases. Therefore, maintaining calcium homeostasis is essential to providing an adequate amount of ionized calcium into the mitochondrial intracellular matrix. Moreover, while mitochondrial calcium signaling can initiate the activity of MSCs, it can also help to restore the cells that were deactivated due to mitochondrial dysfunction.

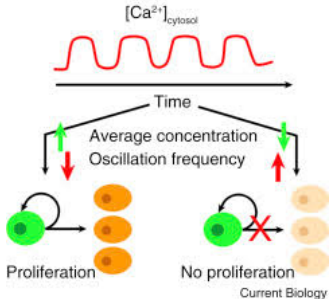


Figure 49. Ca2+ Oscillation Frequencies and Stem Cell Proliferation. Decreased Ca2+ oscillation frequency and increased average Ca2+ concentration in the cytoplasm of Drosophila intestinal stem cells (green) is associated with an increased rate of stem cell proliferation. A reduction in Ca2+ concentration and an increase in Ca2+ oscillation frequency is observed in quiescent stem cells. Adapted from "Intestinal Stem Cells: Got Calcium?," by M. Mászai & J. B. Cordero, 2016, Current Biology, 26, p. 118, Copyright 2016 by Elsevier

Furthermore, Mahajan et al. (2011) have insisted , "calcium deficiency also reduced ($p<0.05$) the in vivo proliferation of MSCs by ~50 %." (p. 373) Insufficient calcium intake reduces MSC proliferation. In an alternative approach, one could say that if the amount of calcium taken is insufficient, the quantity of MSCs generated would increase; thus, it would be useful to specialize the MSCs in specific cell forms, which could be helpful in treating chronic degenerative diseases such as osteoporosis.

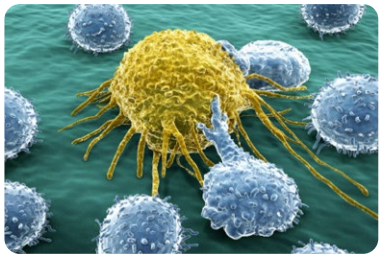
Also, it has been noted that "neonatal Ca nutrition is crucial for bone integrity and suggest[ed] that early-life Ca restriction may have long-term effects on bone integrity via the programming of MSCs." (Mahajan et al., 2011, p. 373) As they suggested, dietary calcium supplements are

critically needed for those who are pregnant, because early-life calcium supplementation is crucial to building bone integrity that is programmed by MSCs. For this reason, SAC can act as a trigger to regulate adequate calcium homeostasis in our bodies, which can be helpful to initiating human-induced MSCs, the key for treating chronic degenerative diseases.

VIII. SAC Therapy and Cancers

- *Systemic Cancer Therapy* -

"Sigma Anti-bonding Calcium Carbonate: By Regenerating Mitochondrial Function and Regulating Lactic Acid or Ammonia Secretion, It Can Efficiently Recover p53 Function, Which is the Key to Treating Cancers."



"In August 2010, a friend of mine came to the institute. He had prostate cancer that had metastasized, and also lived with advanced colorectal cancer. He said that he had just written a will with his lawyer and was on his way back. The attending doctor suggested that he take more calcium to aid his bones, so he came to buy calcium supplements. Although I didn't know what to say to comfort him, I gave a bottle of SAC Calcium and explained how to take it. The next day, he called; he said that after taking SAC Calcium, a preexisting urinary condition had greatly improved. After that, he continued to take SAC Calcium, and a tumor marker examination also confirmed that PSA and CEA values returned to normal after 14 months. This is our first SAC Calcium success story and case study in Canada for someone who was in the terminal stage. Today, ten years later, he is still alive and healthy."

"One day in November 2013, I received a call from a friend of Ms. Molly Holt. Ms. Holt was diagnosed with multiple myeloma (MM) and received anti-cancer treatment, but her physical condition deteriorated. She had gotten worse and had no way of trying alternative repeat chemotherapy.

Our research team recommended this person take SAC Calcium. After which, she continued to take SAC Calcium and her health recovered in the second year; with a cancer recovery prognosis being given in the spring of 2015. That summer, she visited our research lab in Vancouver and thanked us."

1. Introduction

According to WHO (2018), cancer is one of the top ten leading causes of death in high-income countries. As dietary habits and lifestyles have changed, the number of developing chronic degenerative diseases is increasing. Therefore, it is crucial to pay careful attention to preventing the development of cancers at an individual level. It has been reported that 8.8 million people died due to tumors in 2015 worldwide (WHO, 2018), and the global burden of cancer exceeds USD 1.16 trillion. (EI, 2010) Also, approximately 14.1 million new cases of the disease occurred globally in 2012 (EI, 2010). In Canada alone, about 206,200 new cases of cancer were diagnosed, and 80,800 fatalities caused by the disease were reported in 2017. (EI, 2010)

Current cancer treatments include surgical operation, chemotherapy, radiation therapy, immunotherapy, targeted therapy, hormone therapy, stem cell transplant and precision medicine. Even though there are a number of cancer treatment options, none of them has shown a significant impact in conquering cancer. According to Pignon et al. (2000), only four percent of tumor cancer patients survived through chemotherapy. Also, Juan et al. (2009) mentioned that lung tumor patients remain in poor condition, despite the use of radiation therapy. The reason being that current cancer treatment options only focus on decreasing the number of pre-existing tumor cells. Caley & Jones (2012) mentioned that chemotherapy concentrates on the number of tumor cells by interfering

with tumor cell division. However, to treat the tumor, we must understand the underlying mechanism of the proliferation of cancer cells in the human body to move closer to an actual cure.

Mutated cells will continue duplicating if the body's overall metabolism and functions of the cell are not normalized. It is crucial to develop new cancer treatment technology that restores the entire body's mechanisms and regenerates cellular function, which eventually prevents the subsequent proliferation of tumor cells. Therefore, SAC can be utilized to regenerate malfunctioning mitochondria and endoplasmic reticulum (ER), which ultimately prevent possible protein mutation, such as tp53. By stimulating hormone secretion that plays a critical role in calcium homeostasis and regulating the influx and efflux of calcium ions in the mitochondrial intracellular membrane, we can finally trigger the apoptosis of cancer cells by efficiently releasing apoptosomes, such as cytochrome c.

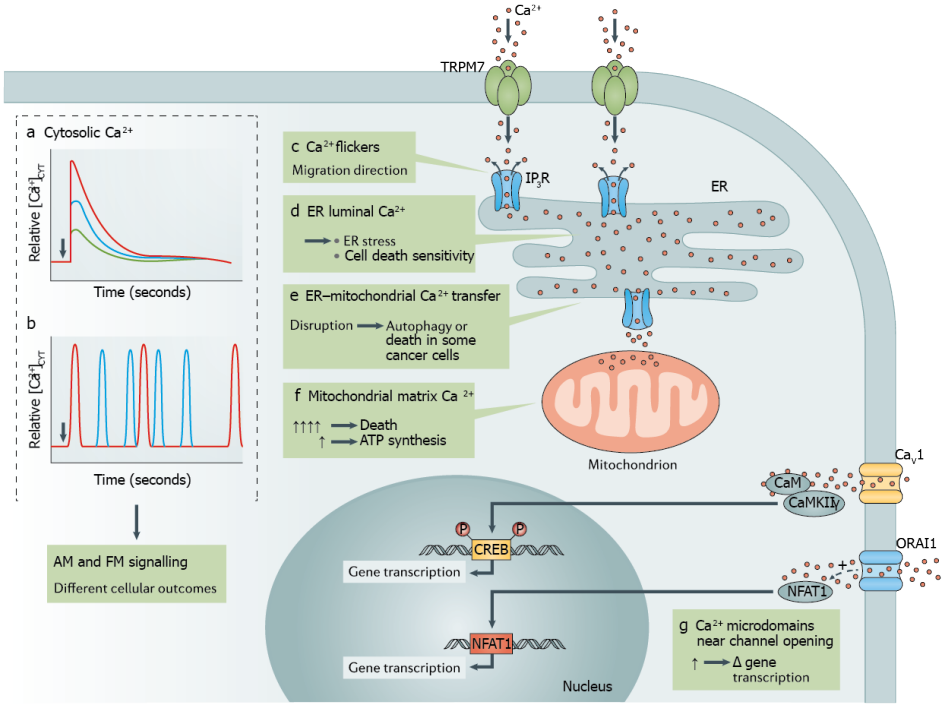


Figure 50. Ca2+ Signal Diversity. This figure illustrates the diversity in Ca2+ signaling and how this may contribute to processes that are relevant in the context of cancer progression. This diversity and its consequences include, but are not limited to, the examples shown. a | The degree of increase in cytosolic free Ca2+ ([Ca2+]CYT) (amplitude modulation (AM)), which may control cell fate (for example, proliferation or cell death); the different colored lines on the graph represent cytosolic Ca2+ increases of different amplitude. b | The frequency of [Ca2+]CYT oscillations (frequency modulation (FM)), which may control the nature of gene transcription and hence different cellular outcomes and expression remodeling; the different colored lines on the graph represent cytosolic Ca2+ increases of varying oscillation frequency. c | Highly localized and transient increases in Ca2+ at the edge of some cells (Ca2+ flickers), which control directional migration. d | Free Ca2+ levels within the endoplasmic reticulum (ER) that may influence cell stress and the sensitivity to apoptotic stimuli, which act in part through the release of Ca2+ from internal stores. e | The confined transfer of Ca2+ from the ER to the mitochondria that, when reduced, may induce autophagy or even death in some cancer cells. f | Levels of Ca2+ within the mitochondrial matrix, where modest changes may promote ATP synthesis, but sustained high levels may promote cell death. g | Localized, very high levels of Ca2+ (known as Ca2+ microdomains) achieved upon the opening of plasmalemmal Ca2+ channels can specifically regulate gene transcription pathways. In some cells, Ca2+-bound calmodulin (CaM) produced near the opening of CaV1 L-type voltage-gated Ca2+ channels can be delivered by Ca2+/calmodulin-dependent protein kinase II subunit γ (CaMKIIγ) to the nucleus to promote the phosphorylation of cAMP responsive element-binding protein (CREB) and gene transcription; in other cellular systems, localized Ca2+ increases produced upon the opening of calcium release-activated calcium channel protein 1 (ORAI1) channels is sufficient via a series of signaling pathways (indicated by the small dashed arrow and the plus symbol) to produce the translocation of nuclear factor of activated T cells 1 (NFAT1) to the nucleus. In contrast, ORAI1-activated gene transcription via NFAT4 also requires increases in nuclear Ca2+ (not shown on this figure). Δ denotes changes in a pathway or levels, ↑ denotes an increase in levels or activity. IP3R, inositol 1,4,5-trisphosphate receptor; TRP, transient receptor potential. Adapted from "The calcium–cancer signaling nexus," by G. R. Monteith, N. Prevarskaya & S. J. Roberts-Thomson, 2017, Nature Reviews Cancer, 17, p. 370, Copyright 2017 by Macmillan

2. Lactic Acid Production in Cancer Cells

"It is possible to monitor the cancer status of cancer patients by measuring the lactic acid decomposing enzyme (LDH, lactate dehydrogenase) in the blood. This is because the lactic acid secreted in cancer cells will be active. SAC Calcium promotes cell normalization and reduces blood lactic acid function."

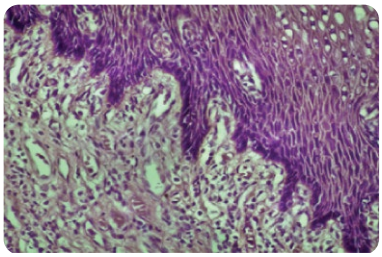


Figure 51. Cancer Cell Infiltration

Healthy cells use both cellular respiration and anaerobic glycolysis by means of mitochondria. Through oxidative phosphorylation, they produce 36 ATP per one mole of glucose and also produce 2 ATP per one mole of glucose when they have an insufficient amount of oxygen for respiration.

However, tumor cells produce lactic acid via aerobic glycolysis, that is a unique metabolism which does not use oxygen during catabolism. (Heiden, Cantley & Thompson, 2009) Tumor cells utilize aerobic glycolysis in order to build physiological constructing elements for cancer cell proliferation. To explain the mechanism in detail, tumor cells require increased glucose uptake for rapid proliferation. Therefore, they use inefficient cellular respiration. Moreover, by using aerobic glycolysis, they can produce more acidic products (lactic acid), which creates a favorable environment for cancer cells. Furthermore, they utilize the biosynthesis pathway for producing palmitate. (Carta et al., 2017)

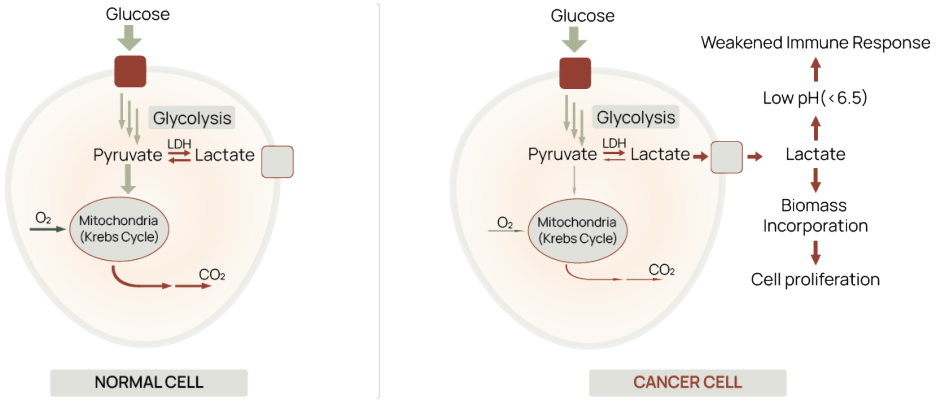


Figure 52. Lactic Acid Production from Cancer Cells

Han et al. (2013) have elucidated that the excessive amount of lactic acid production via cancer cells would activate the secretion of proteases; which causes extracellular degradation, leading to metastasis of cancer cells. Moreover, the excessive secretion of lactic acid would increase the synthesis of HIF-1 proteins, which are actively involved in the proliferation of cancer cells by invading and metastasizing the healthy cells. (Han et al., 2013)

Therefore, it is crucial to neutralize the acidic environment in the cell, and as to how to do this, we suggest SAC therapy. We make this suggestion because as the calcium ions in SAC therapy react with the lactic acid in the cell, it produces calcium lactate, which is the neutral form of salt. Since cancer cells favor an acidic environment (as it interferes with the activity of natural killer cells such as T-lymphocytes and NF-kB cells), by forming calcium lactate, the viability of cancer cells would decrease.



Figure 53. Calcium Lactate Bonding Structure

According to Cooper et al. (1992), patients suffering from lactic acidosis

showed a lack of ionized calcium concentration in the intracellular membrane. Moreover, Gupta (2016) argued that cancer cells use aerobic glycolysis, which "[increases] degradation of glutamine [and] leads to metabolic acidosis with [a] pH of the solid cancers as low as pH 6.0 - 6.5." (p. 1) In other words, a lack of calcium ions causes an increase in the presence of lactic acid in the cellular membrane.

Therefore, supplementing the calcium concentration at an intracellular level is critical to stabilizing an acidic pH level. Strictly speaking, adequate calcium intake is highly recommended for those who are suffering from cancer.

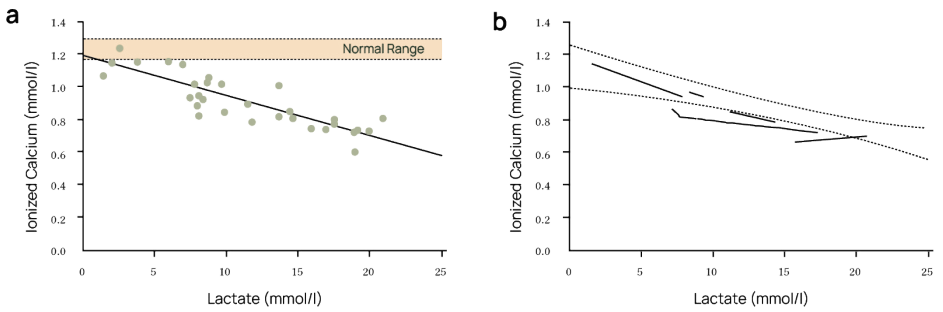


Figure 54. Relationship Between [Ca++] and Plasma Lactate Concentrations in Nine Critically Ill Patients Who Had Metabolic Acidosis. [Ca++] decreased as lactate increased. (a) Linear regression analysis for the group ($r2=0.78$, $p<0.001$) (b) Random effects regression and 95% CI for the 6 patients who had 3 or more data points [slope -0.018 (SE 0.005); intercept 1.11 (SE 0.08)]. Adapted from "Plasma ionized calcium and blood lactate concentrations are inversely associated in human lactic acidosis*," by D. J. Cooper et al., 1992, Intensive Care Medicine, 18, p. 287. Copyright 1992 by the Springer-Verlag

Different from the regular calcium supplements that are sold in the market, SAC does not cause severe adverse effects, such as renal failure. Moreover, with its unique structure and efficient absorption method, SAC can efficiently trigger calcium homeostasis in the body. Also, as it effectively triggers calcium signaling with a tiny amount of calcium carbonate solution, it can be highly useful in neutralizing the cellular pH level as it is ingested. To investigate the efficacy of SAC in offsetting cellular pH levels, Korean National Sports University conducted an experiment in 2014. They examined whether SAC would decrease the amount of lactic acid and glucose production in the cell for 20 Korean professional swimmers after rigorous physical training.

The results were evident, as the average lactic acid level for the swimmers notably decreased after orally ingesting 5 mL of SAC twice a day for two weeks. As SAC has shown significant efficacy in reducing lactic acid levels, we could use it for treating cancer, as acidosis is a serious issue among many cancer patients. Moreover, as their personal performance records improved after use of SAC, we can assume that SAC regenerates mitochondrial function, which results in higher ATP production.

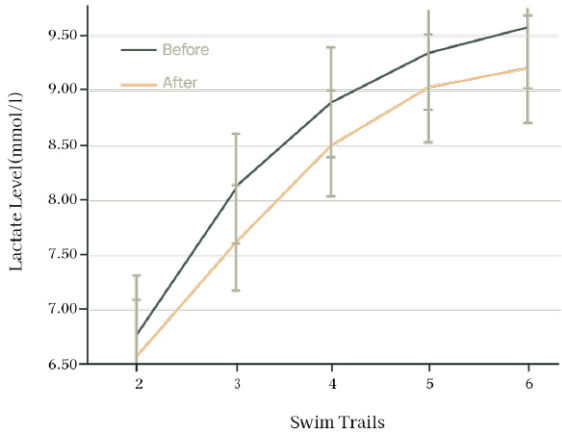


Figure 55. Decreases in Lactic Acid Production After Being Treated with SAC

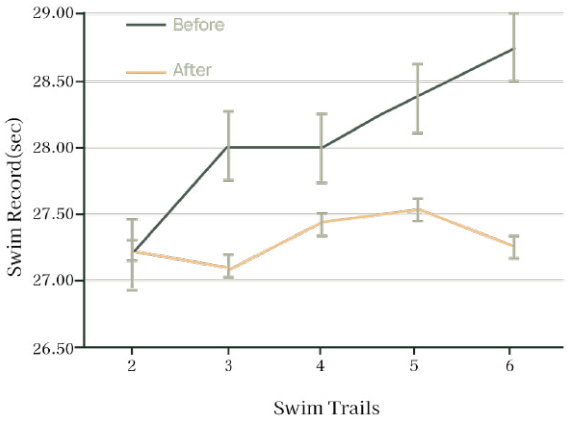


Figure 56. Improvement in Swimming Records After Being Treated with SAC



Figure 57. Pictures Taken When the Research was Conducted

3. SAC and pH (Extracellular and Intracellular)

Dysregulated pH is a common characteristic of cancer cells, as they have an increased intracellular pH and a decreased extracellular pH compared with normal cells. Recent work has expanded our knowledge of how dysregulated pH dynamics influence cancer cell behaviors, including proliferation, metastasis, metabolic adaptation and tumorigenesis. Emerging data suggest that the dysregulated pH of cancers enables these specific cell behaviors by altering the structure and function of selective pH-sensitive proteins, termed pH sensors. Recent findings also show that, by blocking intracellular pH increases, cancer cell behaviors can be attenuated.

This suggests ion transporter inhibition as an effective therapeutic approach, either singly or in combination with targeted therapies. In this Cell Science at a Glance article and accompanying poster, we highlight the interconnected roles of dysregulated pH dynamics in cancer initiation, progression and adaptation. pH, extracellular and intracellular, is dependent on calcium levels. Calcium levels are very important to understanding cancer cell metabolism. Up to 30 % of all people with cancer will develop a high calcium level in blood.

Cancers that more commonly cause high calcium levels in your blood include: lung cancer, head and neck cancers, multiple myeloma, leukemia, kidney cancer, breast cancer, and gastrointestinal (digestive system) cancers, etc.

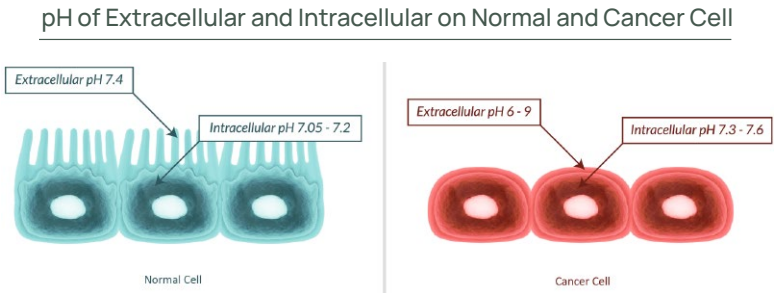


Figure 58. Extracellular and Intracellular PH of Normal and Cancerous Cells

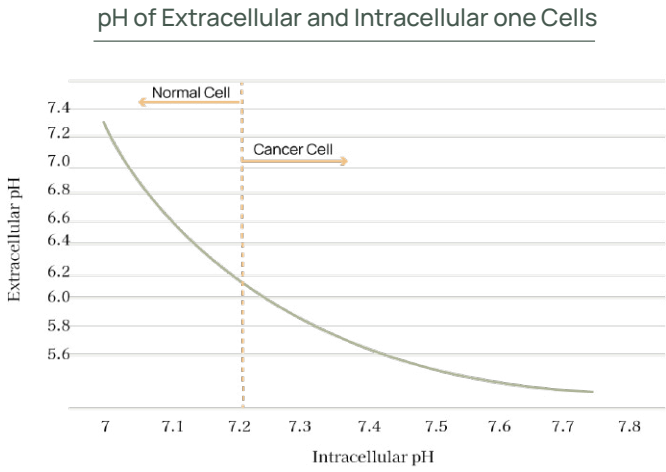


Figure 59. Cancer and pH of Extracellular and iIntracellular pH cells

Hemoglobin is an iron-containing protein in red blood cells that give blood its red color. Hemoglobin makes up about 35 % of total blood content [1] and is known for its oxygen-binding capacity; it carries oxygen from the lungs to tissues and organs, while transporting carbon dioxide from tissues and organs to the lungs. Hemoglobin in the blood is present in two forms, a tense (T) form and a relaxed (R) form. Various factors, such as blood pH and carbon dioxide concentration, determine which hemoglobin form is more favorable under different circumstances. For instance, a low pH and high concentration of carbon dioxide favor the T form, whereas the opposite favor the R form. In the T form, the oxygen-binding affinity of hemoglobin decreases, resulting in a reduced oxygen

supply to target tissues and organs. In contrast, the oxygen-binding affinity of hemoglobin is increased in the R form.

Once oxygen carried by hemoglobin is released in the target tissues and organs, it permits aerobic respiration, which gives cells energy to function. More specifically, oxyhemoglobin is formed when oxygen binds to the hemoglobin in the lungs. The oxyhemoglobin then travels through the bloodstream and releases oxygen at cells where oxygen is utilized for the production of ATP via oxidative phosphorylation.

On the contrary, once oxygen is dropped off, hemoglobin picks up carbon dioxide, a by-product of lipid and carbohydrate metabolism by respiration. It forms a molecule called carbaminohemoglobin, which is accountable for about 10 % of carbon dioxide transportation by hemoglobin. The majority of carbon dioxide molecules (85 %) diffuse into the red blood cells, in which carbonic anhydrase within the red blood cells converts the carbon dioxide into carbonic acid. Moreover, carbonic acid lowers the blood pH, resulting in reduced hemoglobin-oxygen affinity. The correlation between the blood pH level and hemoglobin-oxygen affinity was first proposed by Christian Bohr and is referred to as the Bohr effect, which states that the oxygen-binding affinity of hemoglobin is dependent on blood pH and the concentration of carbon dioxide [1]. Hence, it can be said that blood pH plays a significant role in determining hemoglobin-oxygen affinity, which greatly affects oxygen supply to tissues and organs for aerobic metabolism and energy production, which are essential for cellular survival and functions.

Unlike normal cells, cancer cells metastasize uncontrollably in oxygen-deprived environments due to their ability to maintain and perform cellular functions such as angiogenesis, apoptosis resistance, and proliferation via anaerobic metabolisms, suggesting that subsequent oxygen supply could provide an ideal environment for cancer prevention. Cancer cells occasionally secrete parathyroid hormone-related protein (PTHrP), which is often used in cancer diagnosis as the first sign of malignancy. PTHrP plays a significant role in cancer initiation, growth, and metastasis [2]. Moreover, it is believed that the function of PTHrP is similar to that of parathyroid hormone (PTH) in bone regulation, considering that both PTHrP and PTH stimulate the uptake of calcium in the bones to increase the concentration of blood-calcium concentration, and are capable of

inducing hypercalcemia and osteoporosis via calcium absorption from bones [3].

Moreover, according to Miao et al., osteoblast-specific ablation of PTHrP in mice resulted in osteoporosis accompanied by impairment in bone formation [4]. Hence, it can be said that in the presence of cancer cells, PTHrP secretion increases, which acts on bones and may lead to the development of hypercalcemia and osteoporosis. As the percentage of free ionized calcium increases due to uncontrolled calcium secretion from bones by PTHrP, blood pH decreases (more acidic). Importantly, as mentioned above, as blood pH becomes acidic, hemoglobin and oxygen binding affinity decreases, resulting in the shortage of oxygen supply to tissues and organs. Hence, the reduction in oxygen supply may foster cancer proliferation and metastasis.

Similarly, the binding affinity between calcium and its transporter protein depends heavily on blood pH. In a low pH environment binding affinity decreases, leading to an increased concentration of blood-circulating ionized calcium.

In contrast, in a high pH environment, calcium tends to tightly bind to protein, resulting in a reduction in free ionized calcium levels. It has been proposed that an increased percentage of free ionized calcium due to pH fluctuation could be the body's innate response in an effort to neutralize acidity.

Calcium itself does not promote acidity in the blood but rather is used as a neutralizing factor in fluctuating pH. Hence calcium supplementation may play significant roles in:

1) Preventing osteoporosis caused by excessive PTHrP secretion
2) Neutralizing blood pH and thereby promoting hemoglobin-oxygen affinity for enhanced oxygen supply.

To apply the effects of SAC to the restoration of oxygen supply, we emphasize its outcome on the systemic regulation of hormones associated with calcium homeostasis. It is widely known that significant regulation of calcium homeostasis, and therefore of bone metabolism, is regulated by the secretion of parathyroid hormone (PTH) and thyroid hormone (TH) [5]. SAC initiates systemic regulation of both the PTH and TH involved in calcium homeostasis, which, in turn, stabilizes the absorption and

resorption of bones. Therefore, the systemic control of thyroid hormones by SAC can be efficiently used to normalize blood pH levels caused by excessive ionized calcium circulation in the blood mediated by PTHrP secreted from cancer cells. Hence, taking into account several studies that demonstrated the relationship between calcium regulation in cancer and blood pH levels, the following graph depicts the general concept of oxygenation and calcium regulation (Figure 60).

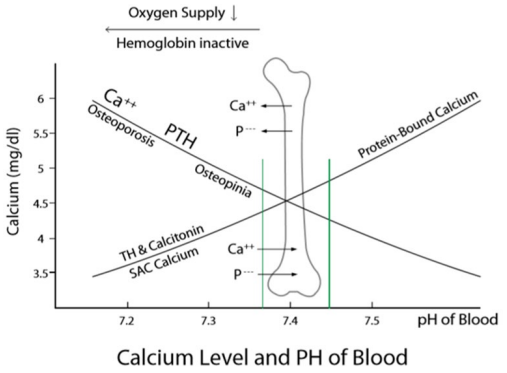


Figure 60. As the blood pH is acidic (pH < 7.4), calcium stored in bones is secreted, and thus increases the blood calcium concentration. Whereas, when the pH level of blood is more basic (pH > 7.4), the concentration of protein-bound calcium increases. Alkalosis refers to the result of a process reducing hydrogen ion concentration in the blood, leading to alkalemia (pH > 7.4). As alkalemia persists, blood transport proteins, such as albumin, become anions and may result in a stronger calcium-albumin affinity, which could lead to a low blood calcium concentration.

Reduction in the supply of oxygen induces the shutdown of oxidative phosphorylation and results in ATP depletion in the mitochondria. The consequent increase in anaerobic glycolysis lowers pH [6]. Healthy cells make use of both cellular oxygen respiration and anaerobic glycolysis via mitochondrial activity. Through oxidative phosphorylation, cells produce 36 ATPs per one mole of glucose, and 2 ATP per one mole of glucose when they have an insufficient amount of oxygen for the reaction. On the other hand, tumor cells produce lactic acid via aerobic glycolysis without requiring oxygen during catabolism [7]. Tumor cells utilize aerobic glycolysis to build physiological construction elements for cancer cell proliferation. Specifically, tumor cells require increased glucose uptake for metastasis. Moreover, by using aerobic glycolysis,

tumor cells can produce more acidic products (lactic acid), making a favorable environment for cancer cell survival and metastasis. Han et al. have elucidated that the excessive amount of lactic acid produced via tumor cells may activate the secretion of proteases, which may cause extracellular degradation, leading to the metastasis of tumor cells [8]. Moreover, the excessive secretion of lactic acid would increase the synthesis of HIF-1 proteins, which are actively involved in the proliferation of tumor cells by invading and metastasizing the healthy cells [8]. Therefore, it is crucial to neutralize the acidic environment in the cell. Tumor cells favor an acidic environment, as it interferes with the activity of natural killer cells such as T-lymphocytes and NF-kB cells. Interestingly, ionized calcium in the SAC solution reacts with lactic acid in the cell, producing calcium lactate, thereby producing an unfavorable condition for tumor cell viability.

SAC has a unique structure and efficient absorption method, enabling efficient calcium homeostasis in the body. Moreover, SAC can effectively trigger calcium signaling and may contribute to the neutralization of pH levels.

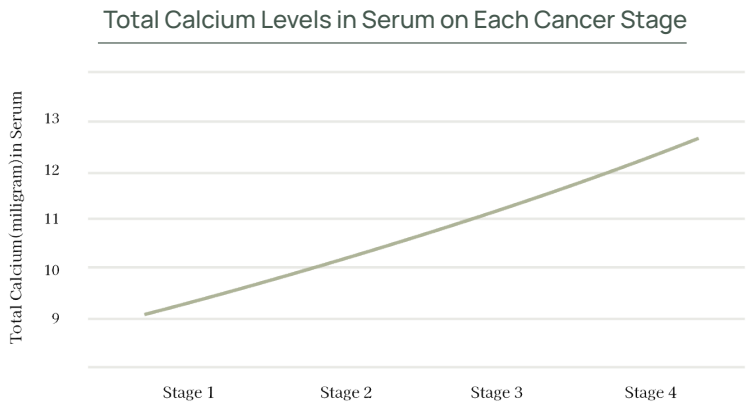


Figure 61. Total Calcium Level in Serum at Each Stage of Cancer

4. Ammonia Production in Sarcoma Tumor Cells

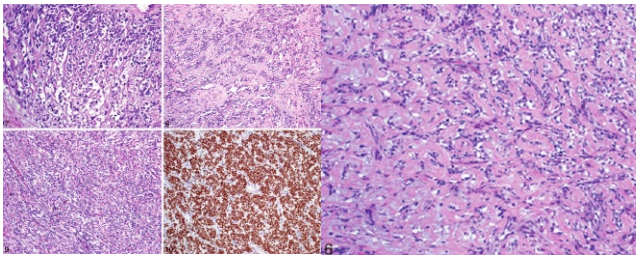


Figure 62. A Kidney's Sarcoma Cell

During sarcoma tumor cell generation, they require a lot of nutrients to form their building blocks. Also, they uptake a massive amount of glucose; as glucose is the primary source of energy that activates their cellular activity. However, other metabolic waste is produced during catabolism, such as ammonia (NH_4). Ammonia is produced through tumor cell metabolism, and with reference to the research conducted by Spinelli et al. (2017), ammonia produces glutamine when they need to obtain energy to metastasize. As Eagle (1955) identified, glutamine is the most utilized nutrient for tumor cells, so repressing glutamine production is the key to inhibiting cancer cell proliferation.

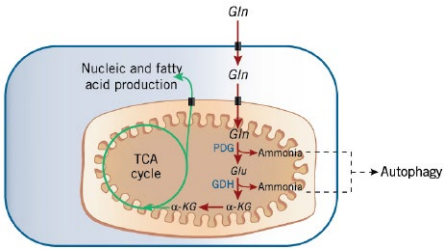


Figure 63. Ammonia as a Stress Signal in Cancer. Ammonia has long been seen as a toxic by-product of glutamine (Gln) metabolism in mitochondria. Adapted from "Sending ammonia signals," by S. Edelson, 2010, SciBX, 3(19), p. 1. Copyright 2010 by Nature Publishing Group.

Furthermore, when the ammonia concentration in the body is too high, the urea cycle is activated in the liver to convert toxic ammonia

into uric acid. (Ali, Gurusamy & Ramachandr, 2014) However, as cancer cells produce an excessive amount of ammonia, the excretory system does not work correctly, which ultimately causes the body to have excess base. (Spinelli et al., 2017) Therefore, as cancer cells are generated, a significant concentration of ammonia remains in the cardiovascular system and extracellular mitochondrial matrix (Tarantino et al., 2009); consequently, this can cause the dysfunction of mitochondria and alteration in protein synthesis. (Niknahad et al., 2017)

According to Spinelli et al. (2017), tumor cells use glutamine to protect themselves from the attack of natural killer cells such as T-lymphocytes and NF-kB Cells. As they repress the expression of thioredoxin-interacting protein (TXNIP), cancer cells can invade healthy cells without interruption. (Morrison et al., 2014) According to Morrison et al. (2014), when they conducted an experiment in mice with thyroid cancer, they found out that TXNIP expression can weaken the growth of the cancer cells. Moreover, as Kaadige et al. (2009) investigated, TXNIP expression is remarkably activated in the presence of glucose. However, it significantly decreased in the presence of both glucose and glutamine. As they concluded that glutamine deactivates the expression of TXNIP (Kaadige et al., 2009), it is critical to repress the production of ammonia, which leads to the mass production of glutamine.

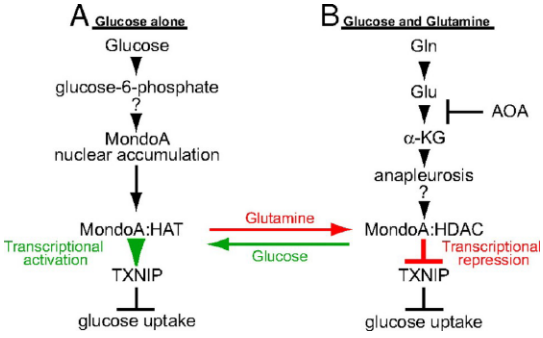


Figure 64. MondoA is a Nutrient-Dependent Transcription Factor. See text for details. As indicated, the mechanisms controlling how MondoA accumulates in the nucleus in response to glucose and how glutamine-dependent mitochondrial anaplerosis converts MondoA to a transcriptional repressor are not known. HAT, histone acetyl-transferase; HDAC, histone deacetylase; -KG, -ketoglutarate; AOA, aminooxyacetate; Gln, glutamine; Glu, glutamate. Adapted from "Glutamine-dependent anaplerosis dictates glucose uptake and cell growth by regulating MondoA transcriptional activity," by M. R. Kaadige et al., 2009, PNAS, 106(35), p. 287. Copyright 2009 by the National Academy of Sciences.

As Dickman, Senozan & Hunt (1970) mentioned, calcium ammoniate is naturally formed as the following reaction occurs; $Ca(NH_3)_6(s) \leftrightharpoons Ca^{2+} + 6NH_3(g)$. They demonstrated that serum ionic calcium could be reacted with 6 moles of ammonia by forming an ionic salt, called calcium ammoniate ($Ca(NH_3)_6$). Therefore, we assume that SAC can be a treatment for cancer by referring to this equation. As SAC significantly increases the serum ionic calcium level, it might also be able to efficiently repress the mass ammonia concentration in the cell. As ammonia produces glutamine, which interferes with the activity of natural killer cells, suppressing the production of glutamine is essential and can be done by causing ammonia to react with SAC.

$$NH_3 + H_2O \rightarrow NH_4(OH);\ pH\ 9\text{-}10$$
$$Ca^{2+} + 6NH_3 \rightarrow Ca(NH_3)_6^{2+}$$
$$Ca^{2+} + NH_4(OH) + H_2O \rightarrow Ca(OH)_2$$
$$4NH_3 + 2(H_2O) \rightarrow$$
$$[Ca(NH_3)_4(H_2O)_2]^{2+} + 2OH^- \rightarrow$$
$$[Ca(NH_3)_4(H_2O)_2](OH)_2$$

Figure 65. Ammonia Inhibition with Calcium in Cancer Cells

5. SAC Triggers p53 Restoration and Apoptosis

It is well-known that p53 is a tumor suppressor that can inhibit cancer cell proliferation and regulate the cell cycle by arresting cellular growth. However, when calcium levels in the body are imbalanced, mitochondria do not work correctly, especially when there is excitotoxicity due to the massive influx of calcium ions into the mitochondrial matrix. This will destroy the mitochondria, which ultimately causes mutations in protein synthesis. In such cases, p53 protein mutates and is converted into the defective p53 protein, which helps the rapid proliferation of cancer cells. As p53/POT1 are mutated and dysfunctional, apoptosis does not occur correctly; therefore, maintaining calcium homeostasis is critical to preventing mitochondrial dysfunction. (POT1: this gene is a member of the telombin family and encodes a nuclear protein involved in telomere

maintenance.)

According to Stanika et al. (2009), calcium excitotoxicity causes mitochondrial dysfunction. Moreover, Murphy (2013) stated that ROS production resulting from the malfunction of mitochondria causes the misfolding of p53 proteins. Also, Hughes, Adam & Gottschling (2012) concluded that the imbalance of pH leads to mitochondrial dysfunction. As mitochondrial dysfunction causes the p53/POT1 mutation, regulating mitochondrial function is critical. While calcium signaling is activated through calcium homeostasis, ionized calcium flows through the calcium ion channel; then it initiates the mitochondrial function.

As Giorgi, Bonora & Pinton (2015) concluded, p53 and POT1 play a role in stimulating calcium signaling in order to activate mitochondrial activity to control oncogenes. However, when p53 and POT1 are mutated, calcium signaling will no longer function properly, which ultimately causes the mass production of ROS, and in turn affects the development of cancer cells. (Macip et al., 2003) Moreover, Kaur & Sanyal (2011) insisted that calcium signaling should regularly oscillate since the imbalance of calcium influx and efflux in the mitochondrial matrix can cause tumor formation. They also added that when calcium homeostasis is adequately regulated, apoptosomes, such as Apaf-1, are produced. (Kaur & Sanyal, 2011)

For this reason, we recommend SAC as a novel form of cancer treatment, as it regulates calcium homeostasis in the body; which initiates mitochondrial activity. Moreover, SAC has proven its efficacy in controlling intracellular calcium ions by regulating pituitary hormones. Therefore, we can assert that SAC can be a treatment option in curing cancer as it neutralizes serum pH levels and regulates mitochondrial activity, which prevents p53 and POT1 mutations and helps to produce apoptosomes.

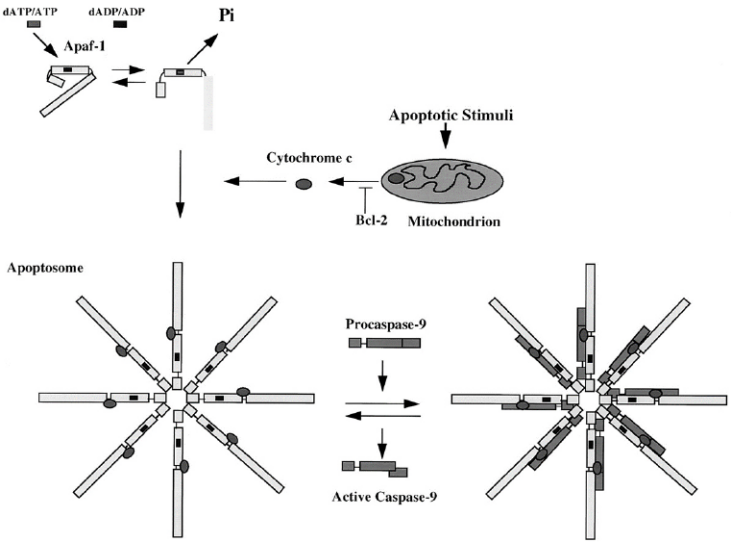


Figure 66. Model of Caspase-9 Activation. See the text for details. APAF-1, cytochrome c, caspase-9, dATP, and dADP are indicated. Adapted from "An APAF-1z Cytochrome c Multimeric Complex Is a Functional Apoptosome. That Activates Procaspase-9*," by H. Zou et al., 1999, JBC, 274 (17), p. 11555. Copyright 1999 by the American Society for Biochemistry and Molecular Biology.

6. Eosinophils and Cancers

6a. The role of eosinophils

To date, although scientists have continued to carry out extensive research on the role of neutrophils and other white blood cells, there are relatively few studies on eosinophils. Therefore, the specific functions of eosinophils in the human body are still unclear. Recently, some studies have been conducted on the functions of eosinophils and cancer cells interchangeably. According to the research results of Davis & Rothenberg (2014), eosinophils play an important role in the immune system's response to cancer cells, specifically indicating that tissue regeneration and cell relaxation are related to homeostasis and disease immune response in the body.

Another name for eosinophils is "acid fats", which are acid-loving immune cells. They are usually observed in vertebrates. Based on current knowledge, they are related to adipocytes and basophils.
The cells together become the cause of the development of allergies and

asthma. In addition, eosinophils are classified as granulocytes found in bone marrow.

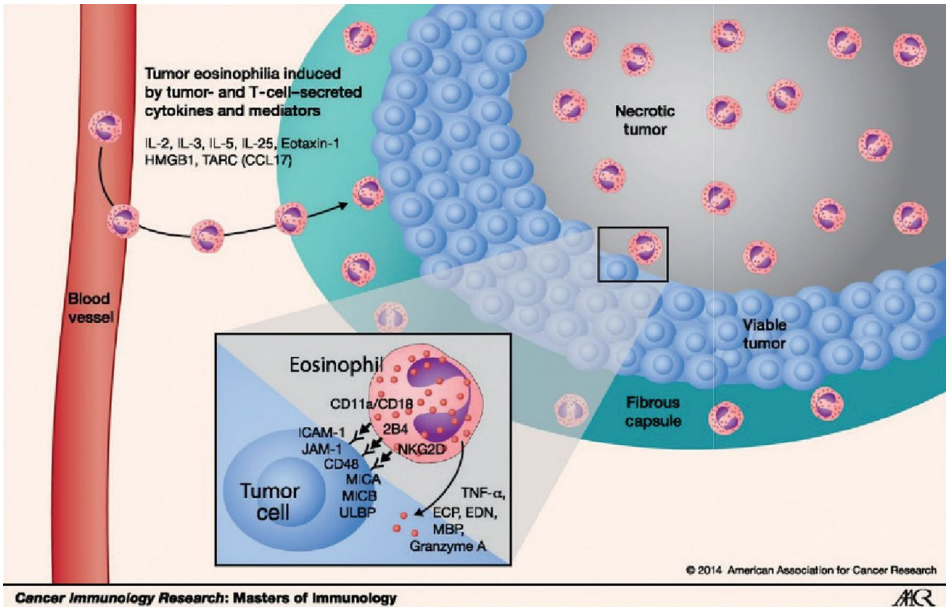


Figure 67. Interaction Between Tumors and Eosinophils. Eosinophils infiltrate all parts of the necrotic fiber capsule of the tumor. Tumor-related cytokines related to eosinophil accumulation are IL-2, IL-3, IL-5, IL-25, GM-CSF, Eotaxin-1 and HMGB 1. Cell contact and cytotoxicity to CD11a/CD 18 and 2B4 appeared, which is considered to be NKG2D. In addition, the particulate proteins ECP, EDN and MBP are involved in the toxicity of tumor cells after they are released from the tumor.

6b. SAC Calcium and eosinophils

Interestingly, our research team confirmed that after administering 0.0012 % sigma anti-bonding molecule calcium carbonate (SAC) to ovariectomized mice for 12 weeks, their eosinophil concentration increased by 500 % (Choi et al. al., 2011).

After systematically studying various experiments and numerous scientific documents, it has been proven that taking SAC solution improves bone quality, cell turnover rate, and eosinophil quality. Therefore, based on the effect of increasing eosinophil counts after the administration of SAC Calcium, we believe that it can effectively block the invasion of foreign cells and the penetration of infectious viruses, and play a role similar

to that of immune cells.

Parameters	Sham	OVX	OVX+SAC
White blood cells (10^3/μL)	4.95 ± 3.15	4.69 ± 1.79	4.84 ± 1.17
Neutrophils (%)	22.28 ± 4.48	23.4 ± 7.18	21.21 ± 6.19
Eosinophils (%)	1.00 ± 0.52[a]	1.16 ± 0.27[a]	5.04 ± 3.88[b]
Basophils (%)	0.04 ± 0.05	0.04 ± 0.05	0.02 ± 0.04
Lymphocytes (%)	73.72 ± 3.97	72.54 ± 6.96	70.36 ± 8.78
Monocytes (%)	2.68 ± 0.78	2.58 ± 0.33	2.50 ± 1.44
Hematocrit (%)	44.16 ± 2.47	45.30 ± 1.23	43.94 ± 2.18
Red blood cells (10^3/μL)	7.95 ± 0.64	8.26 ± 0.24	8.23 ± 0.55
MCV (fL)	55.68 ± 2.11	54.82 ± 0.82	53.44 ± 1.24
MCH (pg)	32.26 ± 1.36	30.94 ± 0.91	30.50 ± 0.75
MCHC (g/dL)	57.94 ± 1.88	56.40 ± 1.33	57.14 ± 0.76
Platelets (10^3/mm^3)	1,440.2 ± 153.80	1,234.8 ± 82.40	1,297.0 ± 91.3

Table 10. MCV; average particle volume, MCH; average particle hemoglobin, MCHC; hemoglobin concentration under the microscope. Sham operation, OVX; no treatment after oophorectomy, OVX+SAC; Sigma anti-bonding molecule calcium carbonate therapy after oophorectomy (By Osteoporosis SAC Therapy on OVX) SHAM: Control group OVX: Mice with ovaries removed OVX+SAC: Feeding SAC to Mice with Ovaries Removed

Specifically, Carretero et al., (2015) reported: "Activated eosinophils must reject tumors in the presence of tumor-specific $CD8^+$ T cells. Cancer hormones occur in the microscopic environment of tumors. [Which is a] substantial change". In order to investigate whether eosinophils induce cell death, they used the "signal-F-monoclonal antibody" to observe a "reduction of eosinophils by inducing cell suicide". As a result, it was discovered that "without eosinophils, tumor rejection response decreased significantly". It can be inferred from this that the increase in the concentration of eosinophils due to the uptake of SAC will lead to the self-destruction of cancer cells. Moreover, Carretero et al. (2015) discovered that the up-regulation of chemokines such as CCL 5, CXCL 9 and CXCL 10 was significantly impaired after eosinophil depletion.

They proposed that "activated eosinophils actually induce the normal-

ization of tumor vasculature and T cell movement by producing cytokines and chemokines." In addition, in order to prove the efficacy of eosinophil therapy as a cancer therapeutic agent, they conducted clinical trials and the results proved that activated eosinophils exhibited anti-cancer activity.

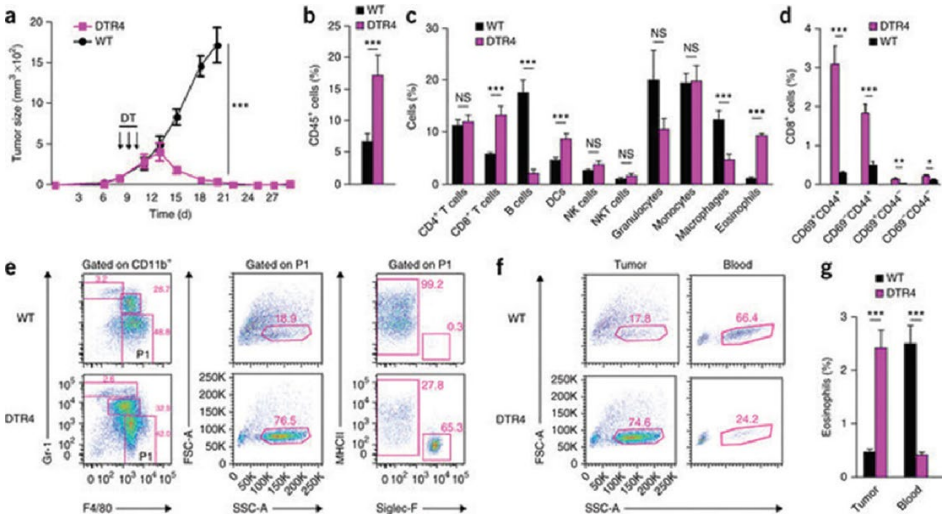


Figure 68. (a) Wild mice treated with diphtheria toxin (DT) on the 8th day after subcutaneous injection of MO4 tumor cells. (WT) and the growth of MO4 tumors in Forxp3.Luci-DTR-4 C57BL/6N mice (DTR 4). For analysis, the tumor was isolated on day 11 (down arrow) and day 13.
(b) On the 13th day after tumor inoculation, in the same mouse, MO4 tumor CD 45+ leukocytes were injected with quantitative diphtheria toxin and analyzed by flow cytometry.
(c) On the 13th day after tumor inoculation, MO4 tumors of specific white blood cell subpopulations (horizontal axis) underwent the same quantification as infiltration quantification (gating strategy, Supplementary Figure 1). DC, dendritic cell; NK, natural killer cell.
(d) As in (a), on the 13th day, the activated state of tumor infiltrating CD8+ T cells in mice is shown.
(e) Flow cytometry of tumor infiltrating cells in mice with CD11b + Gr-1loF4 / 80 + Siglec-F + HCII-FSCloSSChi eosinophilic characteristics. The number adjacent to the area marked by the contour line shows Gr-1hiF4/80- cells (top left), Gr-1hiF4/80+ cells (top right) or Gr-1loF4/80+ (P1) cells (bottom right) (left column). Gr-1loF4/80+ (P1) cells with low-forward scatter and high-side scatter (FSCloSChi) percentage (middle column); MHCII-Sigle-F+P1 cells (left) or MHCII-SSIGF+P1 cells (right) (right column).
(f) Measurement method of cell flow in tumor and peripheral blood of the same mouse. The number adjacent to the contour line indicates eosinophils.
(g) The frequency of eosinophils in the tumor and blood calculated from the results of (e) and (f). NS, inconsequential (P>0.05); *P<0.05, *P>0.01 and *P<0.001" (two-sided dispersion analysis (ANOVA) or unpaired-test (bd, g). The data is from three independent results obtained in one experiment (ad, g, n=average and smm of 6 mice in each group).

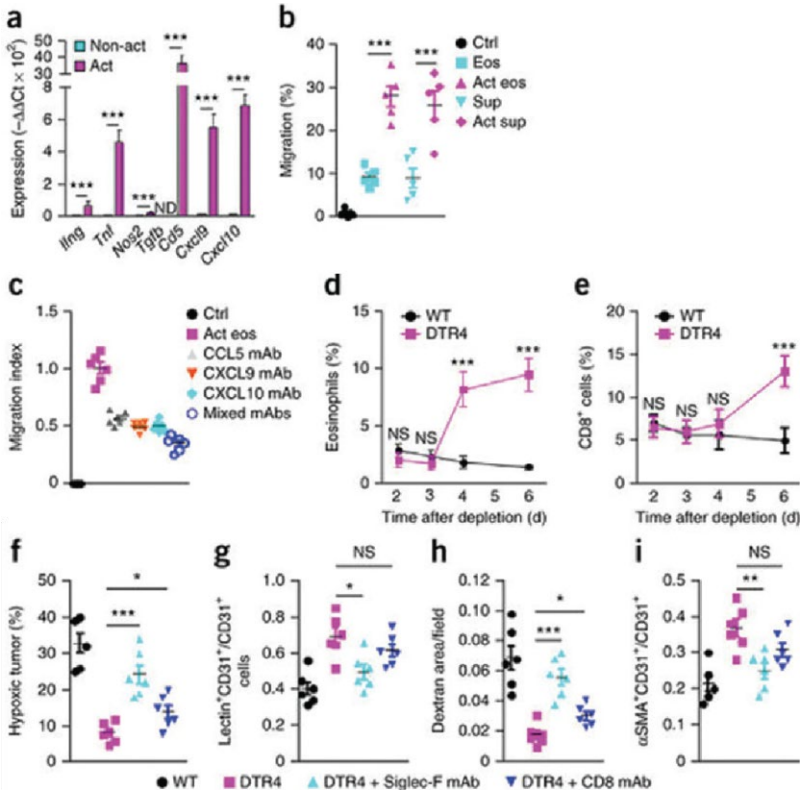


Figure 69. (a) IFN- γ +TNF and in vitro (activation (Act) or inactivation (Non-act)) quantification of mRNA encoding cytokines and chemokines from eosinophils cultured in vitro RT-PCR analysis. ND, not detected.
(b) (Two-chamber system) From the upper cavity, the medium alone (control (Ctrl)), eosinophils (Eos) or activated eosinophils (Act eos) or supernatant containing eosinophils CD8+ T cell migration (Sup) or activated eosinophils (Act sup) in the lower chamber of the fluid.
(c) For single-respiration, activated eosinophils containing CCL 5, CXCL 9 or CXCL 10, or single clonal antibody (mAb) or the lower cavity for a single clonal antibody mixture containing all three (Mixed mAbs). The movement of CD8+ T cells in a system like (b). After tumor inoculation, eosinophils in tumors of wild-type MO4 and Forxp3.LuciDTR.4 mice treated with diphtheria toxin for 8 days (d) and CD8+ T cell
(e) infiltration rate.
(f) The tumors of the mice treated in the same manner as (d) and (e) showed hypoxia, so no monoclonal antibody was injected for Sigle-F and CD8+ (injection combination), evaluated by pemonidazole staining.
(g) Together with the injection of the tomato lectin identified by FITC-, as evaluated by the localization of the adhesion molecule C31 (PECAM-1), the vascular flow from the same mouse tumor was lectin-positive C31+. The cells carry CD31+ cells (lectin+CD31+/CD31+).
(h) The injection of dextran labeled by FITC- is evaluated, as with (f), from the endovascular leakage of the mouse tumor.
(i) Pericyte-endothelial cell association CD31+, which is a mature marker of performance expressed by the ratio of CD31+ cells to α smooth muscle positive C31+ cells (αSMA+CD31+/CD31+), and the localization evaluation of C31. The symbols (b, c, f-i) indicate individual mice. The small horizontal line represents the average (±s.m.m). * P <0.05, ** P <0.01 *** P <0.001 (unpaired t-test). The data are three groups (ae, the average or average of n=5 (a, b) or 6 (ce) mice in each group) or one experiment (fi; each group has 6 mice (fi), in the entire tumor area (f)) or the regional average (g-i) of 10 randomly selected tumors in each tumor.

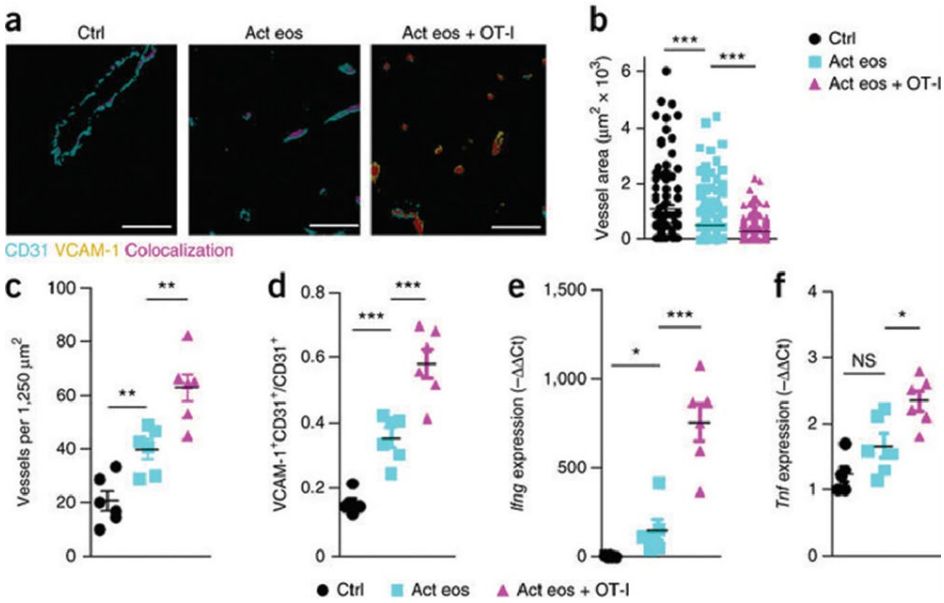


Figure 70. (a) The immunity of CD31 and VCAM-1 in mice (similar in size) without MO4 tumor cells, no additional cells or activated eosinophils, individually or with OT-I cells. Tissue fluorescence was evaluated two days after eosinophils. On the first day after T cell transfer, stain with anti-CD31 (cyan) and anti-CVAM 1 (yellow). Datum ruler, 100μm.(b, c) Tumor vascular size
(b) and tumor vascular
(c) tumor quantification using a.
(d) The ratio of VAM 1-1+C31+ cells to CD31+ cells shows the expression of VCAM-1 in the same CD31+ tumor blood vessels as (a).
(e, f) Just like (a), quantitative RT-PCR analysis of Ifng(e) and Tnf(f) in the lysate of the tumor (as shown in Figure 3a). Each symbol (b-f) represents a single mouse (each group: n=6). The small horizontal line represents the average (±s.m.m). * P <0.05, ** P <0.01, *** P <0.001 (unpaired t-test). The data is obtained from an experiment representing three (ad) or two (e, f) ((bf) each group has n=6 mice average and sem; (c, f) each tumor has 10 regions)

Based on a variety of findings on the effect of eosinophils, we have come to an important discovery in the treatment of cancer an increase in the concentration of eosinophils results from the intake of SAC solution. As we have suggested, eosinophils promote the normalization of tumor blood vessels, strengthen the infiltration of $CD8^+$ T cells, and stimulate the rejection of cancer. In addition, as mentioned earlier, SAC can normalize intracellular calcium homeostasis, thereby regenerating damaged mitochondrial function. Therefore, the absorption of glucose is reduced, and the cancer cells eventually stop proliferating and stop the production of lactic acid. As a result, by regulating calcium homeostasis, we promote the penetration of eosinophils into cancer cells and $CD8^+$ T cells. In reg-

ulating the rejection of cancer, we recommend SAC as a new option for cancer treatment.

7. Current Tumor Treatments

> *"In recent years, most people believe that traditional chemotherapies have failed. As stated in the report "Cancer Etiology" published by the National Cancer Institute (NIC) in 1988, there are divergent opinions that the current many chemotherapy agents are in actuality cancer enhancing or boosting agents."*

7a. Chemotherapy

Chemotherapy is one of the most well-known forms of cancer treatment. It is a cancer pharmacotherapy, so alkylating agents, antimicrotubule agents, and antimetabolites are primarily used for targeting the tumor cells. Sometimes, chemo-hormonal therapies are conducted for those who are suffering from neuroendocrine cancers such as breast and prostate cancer. Mostly, these drugs are DNA recombinants to facilitate efficient apoptosis, and by inhibiting indiscreet mitosis, they can selectively kill the cancerous cells. However, the problem is that there are many different types of tumor cells and various treatment methods are needed to treat cancer effectively. Moreover, as high doses of chemo-therapeutic medicine also alter the function of healthy cells; it can cause many adverse side effects such as myelosuppression, immunosuppression, mucositis, and alopecia. (Lalla et al., 2008; Ockerby et al., 2012; Sengupta et al., 2012)



Figure 71. Current Medications for Cancer Chemotherapy

Therefore, chemo-therapeutic medicine can cause medical conditions such as multiple sclerosis, vasculitis, systemic lupus erythematosus, rheumatoid arthritis, and vasculitis. (Ben-Ari, 2004) According to Partridge, Burstein & Winer (2001), standard short-term and long-term side effects have been observed in breast cancer patients treated with "cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) or methotrexate and 5-fluorouracil (MF) regimens."

Some patients suffered from premature infertility or cardiac dysfunction. Furthermore, chemo-therapeutic medicine can affect the auto-immune system in the body. Therefore, it can cause medical conditions such as multiple sclerosis, vascular cognitive impairment and leukemia after undergoing long-term chemo-hormonal treatment. Consequently, it is crucial to find more natural and holistic treatment options, and we recommend SAC as a novel treatment option for cancer. As it increases the body's metabolism by initiating calcium signaling and produces apoptosomes by modifying mitochondria, it can substitute the role of chemotherapy, meaning that patients with cancer can avoid the possible adverse effects associated with chemotherapy.

Short-term effects	Long-term effects
Emesis	Premature menopause/inferility
Nausea	Weight gain
Stomatitis	Cardiac dysfunction
Alopecia	Leukemia/MDS*
Myelosuppression	Cognitive dysfunction**
Thromboembolism	
Myalgias	
Neuropathy***	
Fatigue***	

*MDS = myelodysplastic syndrome.
**Possible long-term effect; studies are preliminary in nature.
***May be both short-term and long-term effect.

Table 11. Side effects of Chemotherapy. Adapted from "Side Effects of Chemotherapy and Combined Chemo-hormonal Therapy in Women with Early-Stage Breast Cancer," by A.H. Partridge, H.J. Burstein and E.P. Winer, 2001, JNCI Monographs, 96 (8), p. 579. Copyright 2001 by the Oxford Academic

7b. Radiation Therapy

Radiation therapy is also a well-known form of cancer treatment, and it is synergetic when conducted alongside chemotherapy. It is applied to tumors, and it impedes the growth of cancer cells by damaging the DNA of tumor tissues. In use, external beam radiation and x-rays are applied to the tumor cells, and as it breaks down the cancer cells, it will lead to efficient apoptosis to target the malignant tumor cells. However, the problem is that when radiation therapy is applied, physicians should include the margin of normal tissues that are near the tumor cells. By administering high doses of radiation therapy, and as normal cell regions must also be included, it can also kill the DNA of normal tissue, which can exacerbate the poor condition of the human body. Therefore, to avoid possible adverse effects such as infertility, fibrosis, epilation, lymphedema, heart disease, cognitive decline and radiation enteropathy (Meek, 1998; Taylor et al., 2017; "Late Effects of Treatment for Childhood Cancer", 2012; Hauer-Jensen, Denham & Andreyev, 2014), it is critical to find more holistic and palliative treatment options for treating cancer. For this reason, we recommend SAC as a new treatment option for cancer, as it naturally treats cancer by increasing human body metabolism using a tiny amount of SAC solution.



Figure 72. Current Method of Cancer Radiation Therapy

7c. Surgical Operations

A surgical operation is also one of the outstanding treatment options for cancer. Surgeons use scalpels to cut out the infected part of the body to

get rid of tumor cells. They also use anesthesia to reduce pain during surgery. For example, the most frequently performed treatment option for a brain tumor is a craniotomy. By cutting out the infected portion of the scalp, the tumor cells can be removed. However, a surgical treatment has serious side effects, as it can result in permanent disability after surgery. (Kaya et al.,2010) Moreover, Kaya et al. (2010) found that breast cancer patients who had undergone surgical intervention complained of "arm pain on motion, anterior chest wall pain, loss of grip strength, and shoulder flexion." Therefore, SAC can be a new treatment option for treating cancer, as it does not require any surgical procedures. Also, as it enhances overall body metabolism by stimulating calcium homeostasis, it can modify mitochondrial activity, which helps to produce apoptosome enzymes.

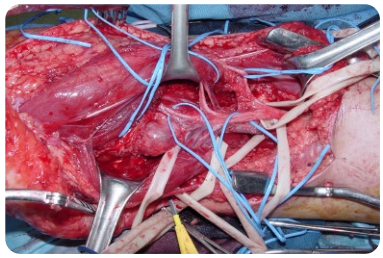


Figure 73. Current Method of Surgical Operation to Remove Tumor Cells

7d. Targeted therapy

Monoclonal antibodies, also known as therapeutic antibodies, are proteins produced in the lab. These proteins are designed to attach to specific targets found on cancer cells. Some monoclonal antibodies mark cancer cells so that they will be better seen and destroyed by the immune system. Other monoclonal antibodies directly stop cancer cells from growing or cause them to self-destruct. Still others carry toxins to cancer cells. Learn more about monoclonal antibodies.

Most types of targeted therapy help treat cancer by interfering with specific proteins that help tumors grow and spread throughout the body. This is different from chemotherapy, which often kills all cells that grow

and divide quickly. The following explains the different ways that targeted therapy treats cancer.

List of Targeted Therapy Drugs Approved for Specific Types of Cancer
https://www.cancer.gov/about-cancer/treatment/types/targeted-therapies/approved-drug-list

7e. Immune and Hormone Therapy

"I met a woman in her early 30s with advanced malignant non-Hodgkin's lymphoma. She was taking chemotherapy at a general hospital in South Korea. The hospital found that the results of chemotherapy had proved ineffective suppressing the cancer cells. So the doctor switched to immunotherapy for her. And increase the volume of the original steroid injection by 7-10 times. In the course of immunotherapy, not only were cancer cells attacked, but also normal cells were attacked, resulting in inflammation and the appearance of autoimmune diseases in the cells.

As for preventative measures, antibiotics and steroids needed to be used in large quantities. The patient's condition was getting worse and one day, the patient's family heard from the attending doctor that they might need to start making funeral arrangements, so they started SAC Calcium therapy as a last resort. Surprisingly, the patient, who could not eat anything, could eat again. Her body temperature, which had dropped sharply, returned to normal. Her bed was moved out of the ICU into the general ward. She could even move around the hospital in a wheelchair, and could even accept visitors. She could also write a letter to her friend as well. But ultimately, all this effort was for naught. The family reverted to immunotherapy once again for the patient. Unfortunately, the patient, who had shown a trend toward recovery, passed away after a week."

Surgery, chemotherapy, and radiation therapy are local or systemic therapies, but immunotherapy can be said to be systemic therapy. Immunotherapy began to be implemented experimentally more than 20 years ago,

until former US President Jimmy Carter used immunotherapy (Keytruda, manufactured by Merck) to cure melanoma and was thereafter accepted by many people as mainstream. It has been reported that KEYTRUDA® has a rate of efficacy of less than 20 %, but when used with PD-1, it has a rate of efficacy of about 60 % on melanoma (Nature Medicine, 2017). Immunotherapy is divided into non-specific immunotherapy and specific immunotherapy. Immune function is strengthened through the use of immune resuscitation agents. As the name implies, the principle of improving immunity and killing cancer cells is adopted. As the general patient is actively receiving treatment, the hospital begins to treat it indiscriminately. The patient receives an injection.

Adoptive immunotherapy mutation of tumor cells, achieving the anticipated therapeutic results with this method is also considered to be difficult.

In addition, hormonal therapy is used as an adjunct and is a treatment method for hormone-dependent cancer. For breast cancer or prostate cancer, the ovaries can be removed to reduce estrogen, while for men, the adrenal glands (testes) can be removed to eliminate testosterone. This is a treatment for removing the diseased breast or prostate cancer. In addition, thyroid hormone suppression therapy is a treatment that controls thyroid cancer by regulating pituitary hormones, but it is not perfect.

7f. The Side Effects of Chemotherapy and Radiation Therawith SAC Therapy

"Oncology specialists say that it is dangerous to use alternative therapy, but there are still patients and their families looking for alternative therapy. Of course, some people choose SAC Calcium. In Canada, at Surrey Memorial Hospital in Surrey, British Columbia, a female patient in her early 50s was receiving chemotherapy for stage 4, colon cancer."

"Since she was taking SAC Calcium at the same time, she recovered much of her physical strength, so she could handle receiving chemotherapy for approximately 2 hour increments, receiving treatment 12 times in total without any side effects from chemotherapy, all while maintaining her health during this period. Unfortunately, due to the side effects of chemotherapy, her thinned intestinal wall appeared to have been perforated by food, and she eventually passed away. SAC Calcium can help restore the fundamental physical strength necessary to be able to handle chemotherapy, but it cannot fundamentally prevent the side effects of chemotherapy. Therefore, CBHI's research team does not recommend taking SAC Calcium and chemotherapy simultaneously. "

For several years, CBHI researchers observed tumor patients who had been treated using chemotherapy and SAC treatment simultaneously. They found that ninety percent of the tumor patients were free of the adverse effects of chemotherapy and radiation therapy with the help of SAC treatment. This is because SAC treatment restores cell viability through regeneration of calcium depolarization by increasing the permeability of calcium in the mitochondrial matrix.

"Once, I received a call from the husband of a 60-year-old woman living in Portland, saying that she had been diagnosed with hemolytic anemia and was treated in the hospital, but her condition was not good, she was only able to sit, It was very difficult to stand. I asked him if he had blood test records and found that most of the patient's blood indicators were significantly lower than normal, and that her LDH value was quite high. I felt that it was a bone-related problem, and guessed multiple myeloma. I hoped that they would be able to visit the CBHI in Vancouver. Soon thereafter, she located the CBHI with the assistance of her husband. At that time, she was pale, as if she was about to faint. I measured her bone density. Her T-score was -4.6, which was obviously lower than the normal value. It was recommended that she dilute 30 ml of SAC Calcium with water 6 times a day, taking it over the course of the day from waking up to before going to bed. After that, she took SAC Calcium for two weeks according to my recommendation. When she came to the CBHI again two weeks later, her face had changed. It was surprisingly radiant. After hearing her vibrant voice in a small coffee shop in Washington state a year later, I was sure she had fully recovered."

8. Summary of Animal Studies: Summary of Animal Studies for Colon Cancer

"*Evaluation of SAC Calcium for Tumor Growth Inhibition in the COLO 205 Human Colorectal Adenocarcinoma Nude Mouse Xenograft Model. June 7, 2017. Charles River Discovery Services.*"

CBHI Canada contracted Charles River Discovery Services, North Carolina (CR Discovery Services, https://www.criver.com) to evaluate SAC Calcium on two dosing schedules for tumor growth inhibition (TGI) in the COLO 205 human colorectal adenocarcinoma nude mouse xenograft model as a feel-good trial for 28 days only.

In the Colo205-e347 study, SAC Calcium was administered orally (p.o.) at 0.83 mg/kg to two groups. One received dosing twice daily, with one dose on the first day (bid to end, beginning day one dose) and the other

received dosing three times daily, with two doses on the first day (tried to finish, beginning day two doses). Treatments started on Day 1 in mice with established subcutaneous COLO 205 tumors. The study included a vehicle control group, dosed bid to end, first day two doses. The study endpoint was a mean tumor volume of 2000 mm3 in the control group or 28 days, whichever came first. The study ended on Day 28. Treatment response was determined from an analysis of the percentage of tumor growth inhibition (% TGI), defined as the percentage difference between median tumor volumes (MTVs) of the treated and control groups, with differences between groups deemed statistically significant at $p<0.05$ using the Mann-Whitney U test. Tumor regression, mean tumor growth, and treatment tolerability also were considered.

In summary, the median tumor volume on Day 28 for the control Group 1 was 936 mm3. Treatment of animals with SAC Calcium at 0.83 mg/kg, was administered bid to end, the first day. One dose and tried to stop, the first day two doses were associated with 0 % and 8%, respectively. Treatments administered in the study were acceptably tolerated based on the group mean BW measurement and clinical observations.

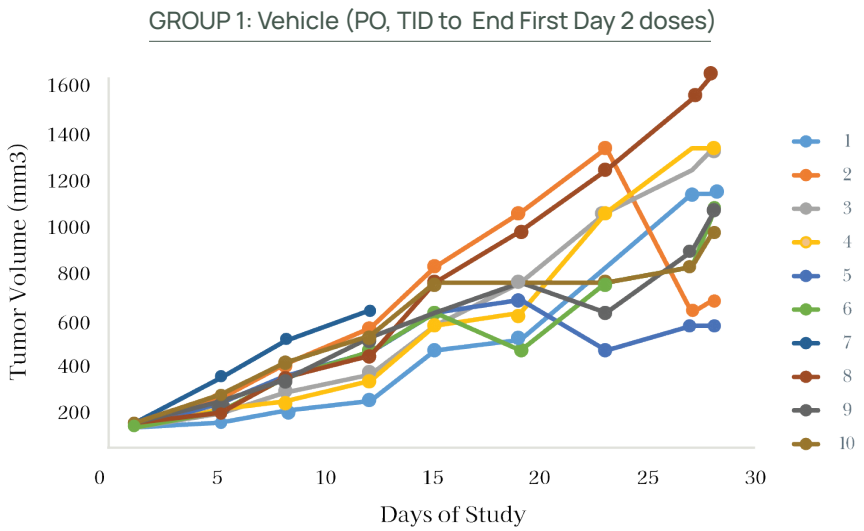


Figure 74. Tumor Suppression After Treat

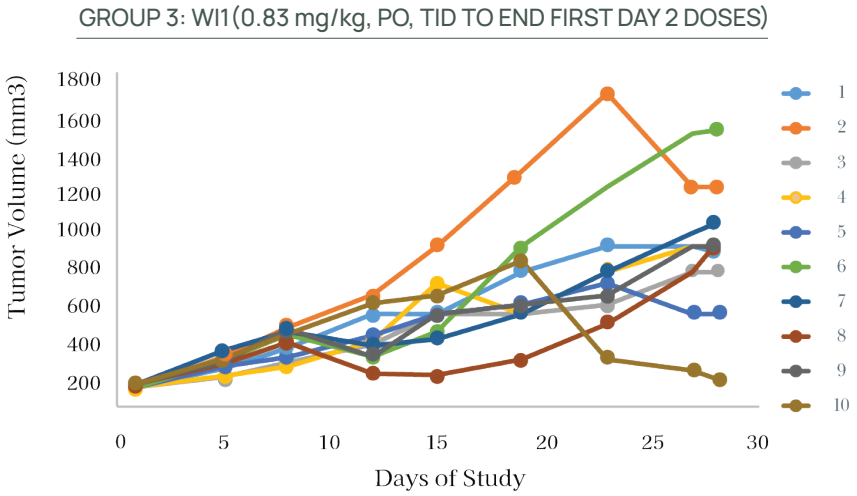


Figure 75. Tumor Suppression After Treatment with SAC (Group 3)

9. Case Study

After treatment with SAC for a certain period of time, patients have shown significant improvement, especially those who are suffering from bone-related cancers. Ms. Holt and Mr. Kim showed notable improvement after treatment with SAC. They have completely recovered from cancer after only being treated with SAC. While the two cases are remarkable, we asked their permission to open their medical data to the public. As is shown in the data, the white blood cell (WBC), hemoglobin, platelet and neutrophil concentrations increased significantly in 4 years. In Mr. Kim's case, the WBC, RBC, neutrophils, lymphocytes and hemoglobin concentrations significantly increased within a month.

9a. Ms. Holt (Multiple Myeloma, 81 years old (2015), Caucasian, Female): Hematological Data Became Normal After Treatment with SAC



Figure 76. Ms. Molly Holt

Date	White blood Cell (WBC)	Hemoglobin (g / dl)	Platelet	Neutrophil
10-25-13	2,590	10.1	79,000	1,350
11-27-13	2,190	9.4	35,000	890
12-10-13	SAC Calcium Start			
12-27-13	3,150	9.9	62,000	1,560
01-21-14	3,650	10.9	89,000	1,710
02-18-14	3,370	11.3	55,000	1,540
03-18-14	3,260	10.8	55,000	1,670
04-08-14	2,670	10.0	67,000	1,270
05-13-14	3,520	9.9	89,000	1,760
06-17-14	3,190	10.0	90,000	1,400
08-05-14	4,160	11.5	101,000	2,160
10-07-14	4,300	10.5	114,000	2,230
12-09-14	4,700	11.2	145,000	2,290
01-27-15	4,760	11.4	152,000	2,560
05-31-17	4,530	12.0	169,000	2,280
09-29-17	4,000	11.9	166,000	2,090
11-27-17	4,190	12.3	171,000	2,100
10-10-18	5,810	12.7	172,000	6,270
Ref	4,000 ml ~ 10,800 ml	M: 13~17 g/dl F: 12~16 g/dl	150,000 ml ~ 400,000 ml	2,000 ml ~ 7,000 ml

Table 12. Changes in Patient's' Hematologic Data After Treatment with SAC

Date	T Score	State
11-25-15	-0.9	Normal
11-20-13	-4.5	Severe Osteoporosis

Table 13. BMD Change for Ms. Holt After Treatment with SAC

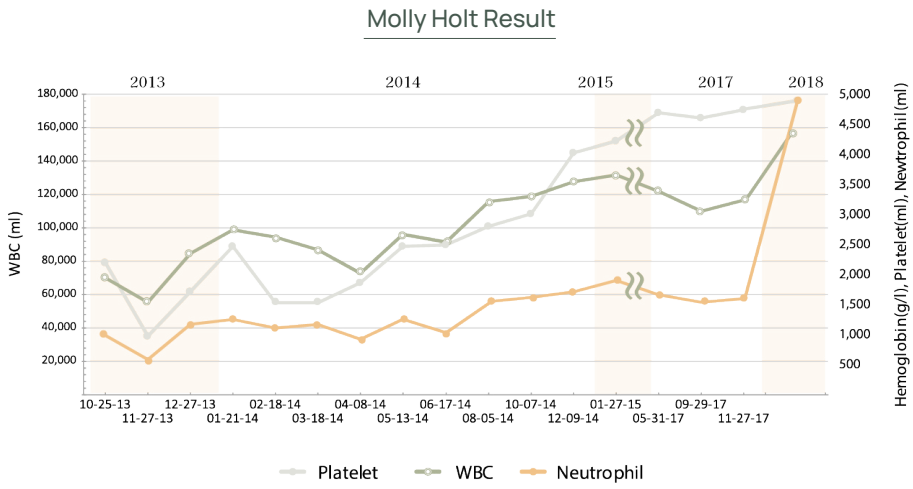
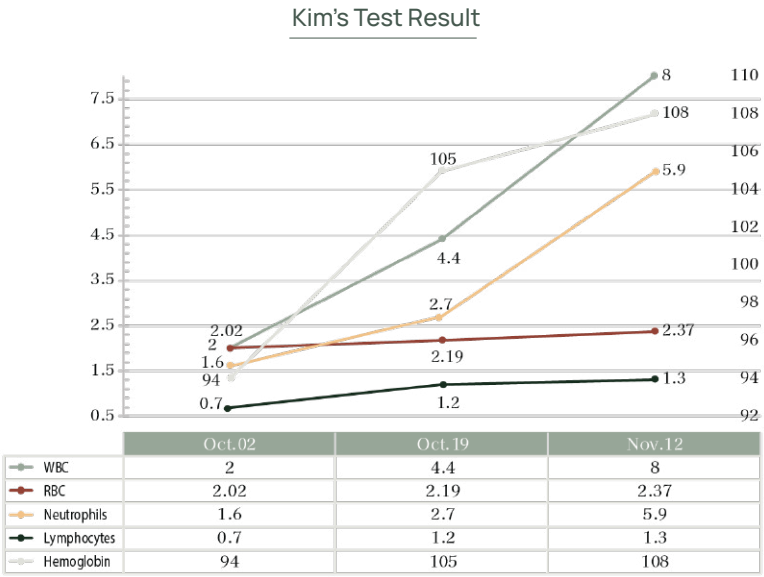


Figure 77. Graphical Change of the Patient's Hematologic Data After Treatment with SAC

9b. Mr. Kim (Chronic Leukemia, 80 years old(2013), Asian, Male, BC, Canada): Hematological Data Became Normal After Treatment with SAC



	Oct.02	Oct.19	Nov.12
WBC	2	4.4	8
RBC	2.02	2.19	2.37
Neutrophils	1.6	2.7	5.9
Lymphocytes	0.7	1.2	1.3
Hemoglobin	94	105	108

Figure 78. Graphical Change of the Patient's Hematologic Data After Treatment with SAC (Dose: 2 times a day; 5 mL with 500 mL of Water)

9c. Cancer Remission under SAC Therapy

Mr. J Crook (Age 66)
Diagnosed with Prostate Cancer
with Liver and Bone Metastasis (2018)

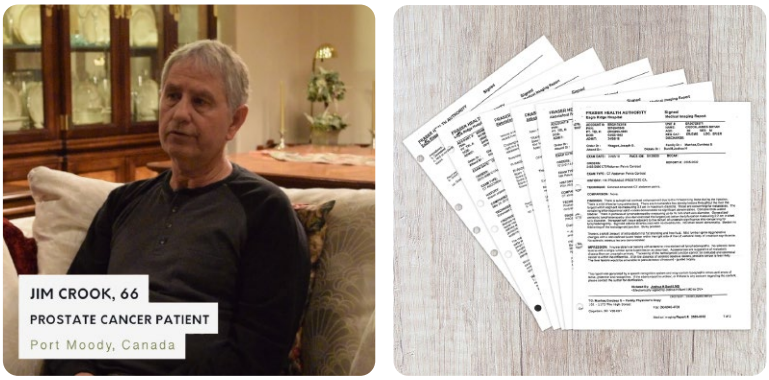


Figure 79. Diagnosis, Doctor's Opinion (June 18, 2018)

Pleuritic Chest Pain, Query: Metastatic Disease

Technique:
Unenhanced Volumetric Axial Images were Obtained through the Chest with Standard Reformats

Findings:
There are several enlarged left supraclavicular lymph nodes, with the largest measuring 1.7 cm in size.
There are fairly extensive coronary artery calcifications.
The visualized upper abdomen gives repeated evidence of numerous masses throughout the liver.

Repeated evidence of multiple enlarged upper abdominal lymph nodes.

Impression:
Left supraclavicular lymphadenopathy, considered to be metastatic in nature.
No other findings of intrathoracic metastatic disease.

Post SAC
Results
(Oct 26, 2018)
Exam Type:
CT Abdomen and Pelvis - Enhanced

History:
Known Prostate Cancer with Visceral Metastases

Technique:
Enhanced CT of the abdomen and pelvis with intravenous contrast

Findings: (After taking SAC since July)
The segment 4a lesion has decreased to 1.6 cm from 3.3 cm. Most of the other lesions have also decreased in size. No definite new lesions in the liver.

The prostate gland has decreased in size with no new focal lesions.

Multiple lower retroperitoneal and iliac chain lymph nodes have decreased in size. A lymph node just inferior to the aortic bifurcation has decreased to 10 mm from 23 mm.
No definite new lymphadenopathy.

IX. SAC-SG Cancer Therapy

- Targeted Cancer Therapy -

1. What is SAC Calcium Bound Glucose, SAC-SG?

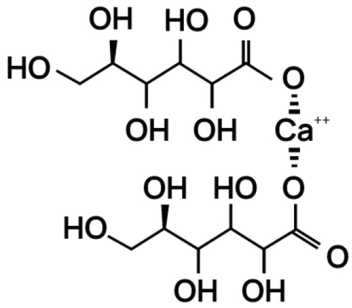


Figure 80. SAC Calcium - Glucose

It's important to understand that cancer cells also require calcium ions for proliferation. As a result, cancer cells secrete PTH-like substances and activate osteoclasts to obtain calcium from bones. Cancer cells access calcium through both ligand-gated and voltage-gated calcium channels, with the majority being obtained through ligand-gated channels. Calcium signals play a crucial role in tumor cell proliferation, and the excess calcium is obtained through

Cancer cells require a large amount of glucose for proliferation, which is obtained through glucose transport (GLUT).

Most of the sugar metabolism is synthesized through aero-glycolysis, a component of cancer cells that provides nucleic acids and amino acids. At this time, the energy source is derived from fatty acids.

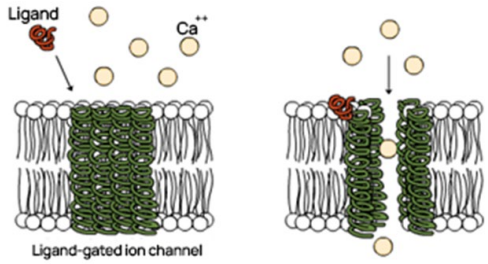


Figure 81. Ligand gate calcium channel and Calcium ion in the cell.

CBHI has developed SAC-SG, a unique material made using the anti-bonding molecule technology of SAC and glucose and calcium. In tumor cells, the SAC-calcium bond with glucose dissociates to produce free radicals in low oxygen conditions, leading to the destruction of tumor cells through strong oxidative power, specifically targeting the metabolism of tumor cells in low oxygen environments.

An important characteristic of cancer cells is the increased uptake of glucose. GLUT (Glucose Transporter) is a crucial target in cancer treatment because cancer cells upregulate GLUT, a membrane protein that facilitates the basic uptake of glucose in most cell types, allowing for the flow of sugar into metabolic pathways.

Cancer cells have the ability to absorb a large amount of glucose, including SAC-SG, 200-300 times faster than normal cells, depending on the location or characteristics of the tumor. A PET (Positron Emission Tomography) scan uses this property to diagnose cancer by binding the fluorine isotope (Fluoro-Deoxyglucose-18) to sugar.



Figure 82. PET (Positron Emission Tomography) CT Scanner

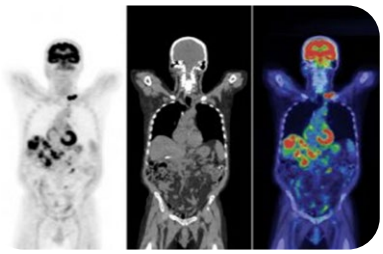


Figure 83. PET (Positron Emission Tomography) CT Scanned Images

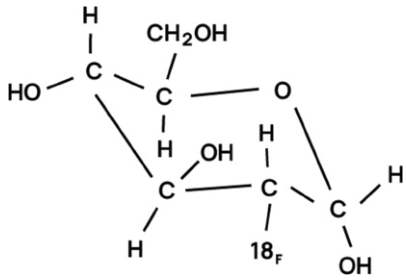


Figure 84. Fluoro-deoxyglucose-18 (2-deoxy-2-[18F]fluoro-D-glucose)

1a. SAC Cell Absorption Trial Using GCaMP on Zebrafish

GCaMP is a genetically encoded calcium indicator (GECI) initially developed in 2001 by Junichi Nakai. It is a synthetic fusion of green fluorescent protein (GFP), calmodulin (CaM), and M13, a peptide sequence from myosin light-chain kinase. When bound to Ca^{2+}, GCaMP fluoresces green with a peak excitation wavelength of 480 nm and a peak emission wavelength of 510 nm. It is used in biological research to measure intracellular Ca^{2+} levels both in vitro and in vivo using virally transfected or transgenic cell and animal lines.

SAC (Sigma Anti-bonding Calcium Carbonate) Study with GCaMP on Zebra Fish by Prof. Sandra Rieger, Sylvester Comprehensive Cancer Center, Univ. of Miami, Jan 2021

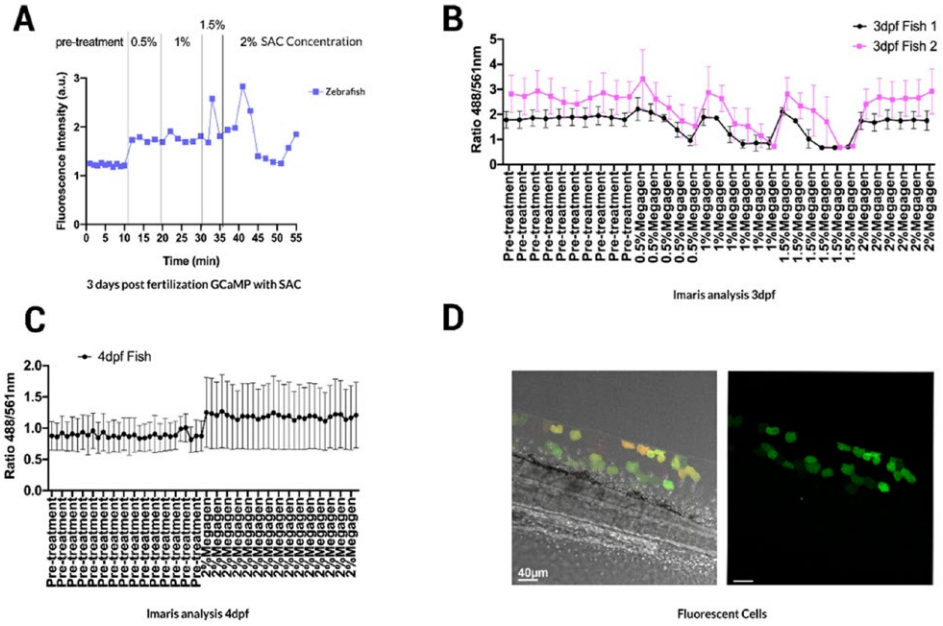


Figure 85. SAC Cell absorption trial using GCaMP on Zebrafish

Fig 85. In the figure, D depicts the fluorescent cells which were analyzed. Zebrafish transient microinjections resulted in a mosaic expression; therefore, only some cells expressed the GCaMP. A red fluorescent transgene was co-expressed as background control. In B, which is the date from the 3dpf fish, each in a different color.

Cells were individually measured and combined as mean +/- SEM. The ration of Green (GCaMP fluorescence) was calculated at 488 nm; 561 nm (dsRed fluorescence). Based on the fluorescence ratios there were spikes at 3 days post fertilization (dpf) in the fish after the treatment concentration was increased. The results are consistent with the expectation. After analyzing the data, there seemed to be a clear response at SAC 0.5 %, 1 %, 1.5 %, 2 % within 2-3 min.

1b. Anti-Cancer Mechanism of each chemicals

Metabolism	Compound	Target	Action
Glycolysis	Phloretin	GLUT1	Inhibits glucose uptake
Glycolysis	WZB117	GLUT1	Inhibits glucose uptake
Glycolysis	2-Deoxyglucose	Hexokinase	Inhibits glycolytic flux
Glycolysis	3-Bromopyruvate	Hexokinase	Inhibits glycolytic flux
Glycolysis	Lonidamine	Hexokinase	Inhibits glycolytic flux
Glycolysis	FX11	Lactate dehydrogenase	Inhibits pyruvate metabolism
Glycolysis	Oxamate	Lactate dehydrogenase	Inhibits pyruvate metabolism
Tricarboxylic acid cycle Dichloroacetate	Pyruvate	Dehydrogenase kinase	Promotes oxidation in the mitochondria
Fatty acid synthesis	C75	FASN	Inhibits fatty acid synthesis
Targets proteins that control how cancer cells grow, divide, and spread	Avastin Imfinzi etc	Protein	Inhibits of target protein. EGFR, ALK, ROS-1, BRAF,NTRK.MET, RET ETC
Immunotherapy	Ipilimumab,Avelumab,Durvalumab,Nivolumab,Pembrolizumab,Atezolizumab etc	PD-L1.PD-1, CTLA-4, TIGIT, LAG	Inhibits of immune checkpoint PD-L1.PD-1, CTLA-4, TIGIT, LAG

Table 14. Anti-Cancer Mechanism of each chemicals

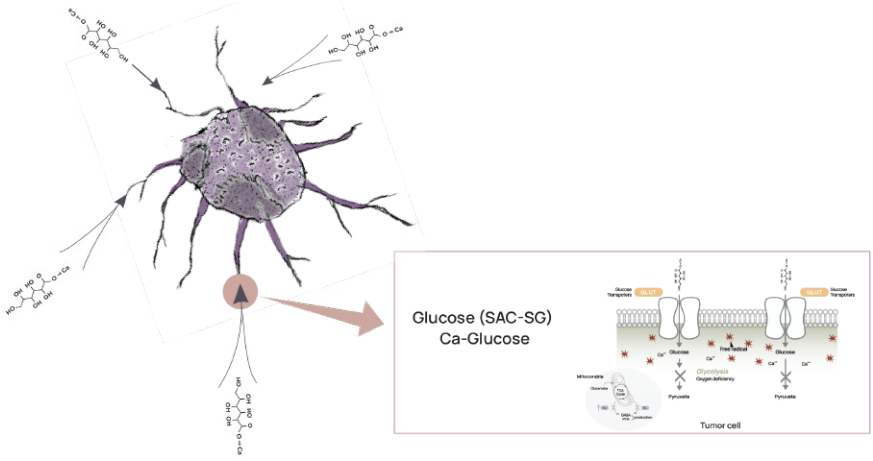


Figure 86. Absorption SAC-SG and oxidation by free radicals in tumor cells. And Process of tumor cell destruction by free radicals of calcium ions(SAC-SG enter Tumor Cells through the Glucose Transport(GLUT)

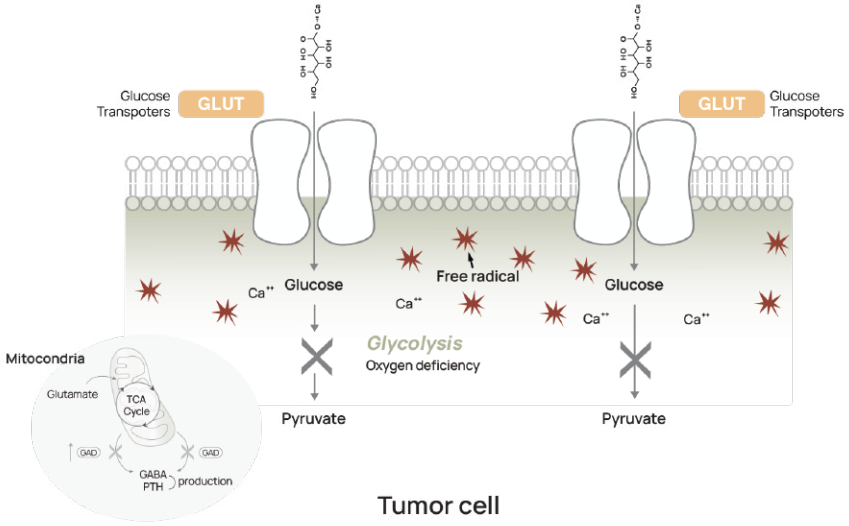


Figure 87. Absorption SAC-SG and oxidation by free radicals in tumor cells(Glucose(SAC-SG) Ca-Glucose)

In tumor cells, the instability of SAC-SG leads to the breakdown of calcium-glucose into free radicals during the aero-glycolysis process through oxidation.

Free radicals are molecules with unpaired electrons that can either steal electrons from stable molecules or donate electrons. The loss of an electron causes the molecule to become oxidized and become a free radical itself.

The generation of free radicals through oxidation in tumor cells can result in a chain reaction of molecular, genetic, and cellular changes that cause permanent damage, making oxidation a significant negative factor for tumor cells.

In addition to glucose, tumor cells also need potassium and sodium. They use calcium, phosphorus, glutamine, citric acid, and fatty acids for proliferation, with the ratio of calcium and phosphorus being crucial. Most of the calcium and phosphorus come from the bones, which can result in hypercalcemia and make bones vulnerable to metastasis in cancer patients. SAC-SG is highly effective against tumor cells as a targeted cancer treatment

2. What is SAC-SG metabolism in normal cells?

In oxygen-rich normal cells, SAC-SG's glucose is used as an energy source through the process of glucose -> pyruvate -> ATP + CO_2 + H_2O, and free radicals are not generated.

At this time, ionic calcium neutralizes lactic acid or reduces lactic acid production and facilitates overall cellular metabolism, such as neurotransmission, DNA replication, enzyme activation, and fatty acid metabolism.

The intracellular calcium level due to the ionic calcium produced intracellularly by separation from sugar from SAC-SG is regulated by the intracellular voltage Em = -60 mV, and the ionic calcium entering through the calcium channel is regulated to increase the concentration of calcium in the blood, thereby increasing TSH – PTH Decrease. Eventually, it stops bone resorption and increases bone density.

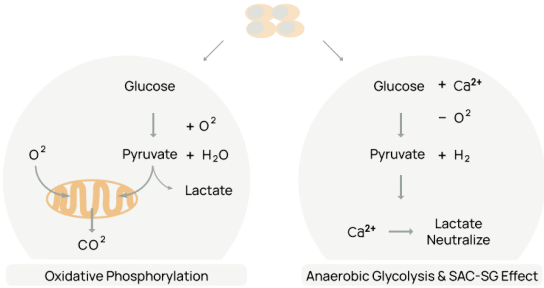


Figure 88. Normal Cell(Differentiated tissue) with SAG-SG

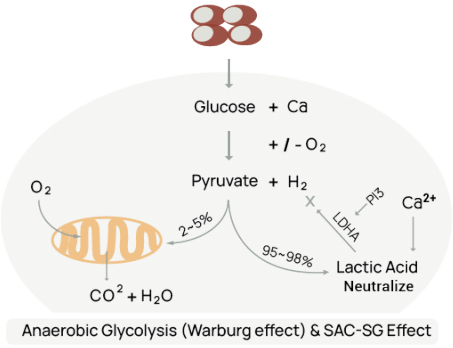


Figure 89. Tumor Cell(Proliferative tissue) with SAC-SG

The low production of ATP in normal cells under oxygen deficiency and low calcium levels generates free radicals, causing oxidative stress. This stress causes the cells to wear out, repair slowly, and malfunction in DNA/p53/apoptosis and DNA mutation, potentially leading to the onset of diseases and premature aging and a high risk of becoming cancerous.

There is an innovative solution that can repair the body at the cellular level, providing a boost in vitality and promoting positive cellular health. SAC-SG is a novel material that triggers the body's natural repair and regeneration processes.

SAC-SG is designed to help reduce oxidative stress damage by enhancing the body's biological signals to fast-track cellular repair. The calcium signal from SAC-SG strengthens the body's own biological signals to

achieve better results.

In aging normal cells, SAC-SG increases the biochemical signals for antioxidant production, which combats oxidative stress caused by free radical damage. This increased signaling pathway helps prevent chronic illnesses and maintains normal cellular function.

SAC-SG and SAC solutions work to counterbalance oxidative stress and have potential benefits for not only treating cancer, but also improving the conditions of individuals with chronic or autoimmune diseases. It also helps maintain normal cell health, slows the effects of cellular aging, improves cell metabolism, and increases energy production (ATP)."

SAC- SG and T-Cell in immunotherapy

Calcium (Ca^{2+}) signaling is of paramount importance to immunity. Regulated increases in cytosolic and organellar Ca^{2+} concentrations in lymphocytes control complex and crucial effector functions such as metabolism, proliferation, differentiation, antibody and cytokine secretion and cytotoxicity.

Altered Ca^{2+} regulation in lymphocytes leads to various autoimmune, inflammatory and immunodeficiency syndromes. Several types of plasma membrane and organellar Ca^{2+}-permeable channels are functional in T cells.

Tumors may continue to grow despite the presence of cancer-killing immune cells. Elevated ionic calcium causes T cells to maintain a stem-cell-like quality, or "stemness," that is closely tied to their ability to eliminate cancer during immunotherapy.

The CBHI suggests that increasing T cells' exposure to calcium or mimicking the effects of high calcium could make cancer immunotherapies more effective.

Immunotherapy has led to remarkable results for some patients' cancers, eradicating difficult-to-treat tumors and, in some cases, causing complete remission of disease. But many patients' tumors do not respond to immunotherapy treatments, and researchers are working to determine why this is.

In addition, some immunotherapy treatments, such as CAR T cells and immune checkpoint inhibitors, are limited by the life span of T

cells. Cancer-fighting T cells inside the tumor can get "exhausted" and die. Therefore, researchers are exploring ways to help T cells used for immunotherapy not only last longer but replicate and grow.

The CBHI team previously suggested that high levels of calcium inside tumors released by dying cancer cells can shut off cancer-killing T cells that have invaded the tumor. In the new suggestion, CBHI assumed that growing T cells under conditions of high calcium also preserves the "stemness" of the T cells.

This means that, in the tumor, the stem-cell-like T cells have the ability to replicate themselves, but they aren't able to mature into killer immune cells. By keeping T cells in this state, the tumors can avoid attack and continue to grow. This could explain how a cancer could grow despite the presence of T cells that would seemingly be able to fight the cancer.

However, when the stem-cell-like T cells are removed from the tumor, grown to large numbers in the lab, and then returned to the patient as they are in a primary immunotherapy strategy called adoptive cell transfer the stem-cell-like T cells can mature into killer cells that can attack the tumor. The preserved stemness of T cells that is, their ability to self-renew indefinitely and respond to stimulation to become cancer-fighting cells may be what allows adoptive cell transfer therapy to be successful.

The CBHI next explored preserving T cells' stemness with high calcium levels for therapeutic use. CBHI assumed that T cells grown in the presence of extra calcium and transplanted into the body with tumor shrank primary and metastatic melanoma tumors better than T cells grown in normal levels of calcium. CBHI also found that in case study, when exposed to a high concentration of calcium, both T cells isolated from patient tumors as well as genetically engineered anticancer T cells had higher levels of markers associated with continued growth and improved immunotherapy outcomes.

Finally, the research team demonstrated indirectly that when they used only SAC Calcium with SAC-SG to get calcium's effects on T cells in cancer patients, this improved the T cells' ability to continue to grow and eliminate tumors. This means that such a drug could potentially be used to induce stemness in T cells as a strategy to enhance cancer immunotherapies.

We are harnessing the stemness capacity of T cells to treat cancer with

SAC Calcium and SG. It's magnificent what happens in case studies. It explains how some immunotherapies work and suggests how we can improve them."

Basic SAC of CBHI research increases our understanding of human behavior and biology, which is foundational to advancing new and better ways to prevent, diagnose, and treat disease. Science is an unpredictable and incremental process each research advance builds on past discoveries, often in unexpected ways. Most clinical advances would not be possible without the knowledge of fundamental basic research.

Cancer stem cells (CSCs) -Tumor initiating cells

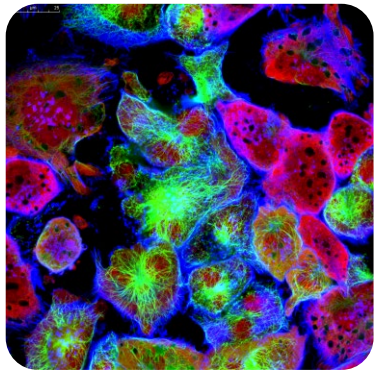


Figure 90. Tumor cells under microscope labeled with fluorescent molecules

Why does cancer recur?

Already in 1877, Virchow's student Cohnheim noticed this cell population and pointed out that it possesses an embryonic character*. Today, those cells are called cancer stem cells (CSCs) or tumor-initiating cells (TICs) and are seen as drivers of tumor establishment and growth, often correlated to aggressive, heterogeneous and therapy-resistant tumors. Upon application of therapeutic regimens such as chemo- or radiotherapy the composition of tumor cell subpopulations changes. At first, tumor cells with a high proliferative capacity are targeted and depleted causing a decrease in tumor size while CSCs survive. Additionally, some tumor cells will become senescent [therapy-induced senescence (TIS)], and

subsequently can cause a change in the tumor microenvironment (TME) with tumor promoting effects due to the senescence-associated secretory phenotype (SASP).

It is well-known that CSCs are resistant to treatment and can cause tumor relapses. However, under the therapeutic pressure and changed microenvironment CSCs can be newly generated. In this case, these cells do originate from non-CSCs or from therapy-induced senescent tumor cells. It is therefore of importance to characterize these cells in detail and to understand their origin at the time of tumor initiation and tumor relapse. Cancer stem cells are estimated to account for 0.1-1% of the total number of cancer cells.

Tumor initiation can either be driven by transformed differentiated cells or transformed tissue resident stem cells. The transformation can take place during tissue regeneration and can additionally, be initiated and/or accelerated as a response to infections, toxins, radiation or metabolic influences causing mutations. During the transformation process, oncogenes are overexpressed and tumor suppressors are inactivated promoting uncontrolled growth of the cell. As a consequence, cells de-differentiate and acquire stem cell characteristics. The transformation of tissue resident stem cells or their progeny is believed to presuppose a different set of genomic changes allowing uncontrolled, niche-independent proliferation. As stem cells already possess unlimited growth potential, it is believed that the transformation of stem cells and their progeny requires only few genomic changes

Recurrence of cancer is mostly due to cancer stem cells. Cancer develops when genes in normal cells of the human body are mutated due to environmental causes. Then, in order for cancer to adapt to various environments and grow and differentiate, cancer cells mutate their DNA again and develop into stem cells so that they can divide to suit various environments.

To date, there is no anticancer drug that can completely kill cancer stem cells (adult cells). 1st-generation cytotoxic anti-cancer drugs, 2-generation targeted anti-cancer drugs, 3-generation immuno-anticancer drugs, and ADC (antibody drug conjugates) target anti-cancer drugs upgraded from targeted anti-cancer drugs are not possible.

The reason is that cancer stem cells have to metabolize to do something

in response to cancer cells, such as monoclonal antibodies or whatever. During chemotherapy, many other cancer cells can be killed by the anticancer drug, but cancer stem cells are still alive and do not metabolize. These stem cells start metabolism again at any time when the conditions are right several years after chemotherapy, and create different cancer cells from the previous one. This is called cancer recurrence. It means that it has DNA completely different from the DNA of the previous cancer and actively replicates again. So, if it recurs, it is often witnessed around many cases where anticancer drugs do not work well and are struggling.

All cancers have their own stem cells that are characteristic of that cancer.

The use of biomarkers in diagnosis, therapy and prognosis has gained increasing interest over the last decades. In particular, the analysis of biomarkers in cancer patients within the pre- and post-therapeutic period is required to identify several types of cells, which carry a risk for disease progression and subsequent post-therapeutic relapse. Cancer stem cells (CSCs) are a subpopulation of tumor cells that can drive tumor initiation and can cause relapses. At the time point of tumor initiation, CSCs originate from either differentiated cells or adult tissue resident stem cells. Due to their importance, several biomarkers that characterize CSCs have been identified and correlated to diagnosis, therapy and prognosis. However, CSCs have been shown to display a high plasticity, which changes their phenotypic and functional appearance. Such changes are induced by chemo- and radiotherapeutics as well as senescent tumor cells, which cause alterations in the tumor microenvironment. Induction of senescence causes tumor shrinkage by modulating an anti-tumorigenic environment in which tumor cells undergo growth arrest and immune cells are attracted. Besides these positive effects after therapy, senescence can also have negative effects displayed post-therapeutically. These unfavorable effects can directly promote cancer stemness by increasing CSC plasticity phenotypes, by activating stemness pathways in non-CSCs, as well as by promoting senescence escape and subsequent activation of stemness pathways. At the end, all these effects can lead to tumor relapse and metastasis.

To explain metaphorically with a tree, the myriad leaves can be compared to normal cancer cells, and the stems and roots to cancer stem cells.

General toxic anticancer drugs, targeted anticancer drugs, and radiation therapy can relatively easily destroy numerous leaves and drop them to the ground, but the roots and stems do not die and someday sprout and grow into leaves again.

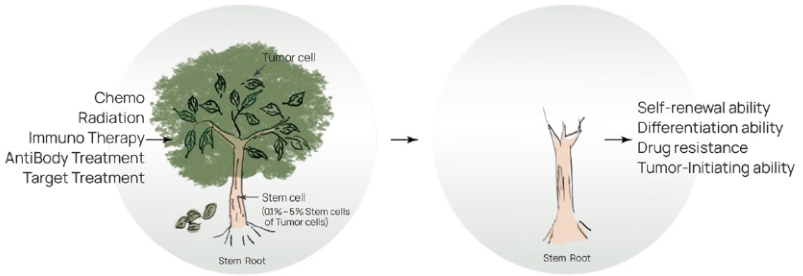


Figure 91. Cancer stem cell analogy of a tree

Fundamentally, sugar, fat, amino acids, and calcium ions are essential for cancer stem cells to proliferate and differentiate. If we make good use of these, we are confident that we can be liberated from cancer recurrence.

Currently, it is judged that the only way to eliminate cancer and cancer stem cells is to increase NK cell effectiveness. This method is the only way to get rid of cancer and cancer stem cells without side effects, and it is also a way to prevent cancer recurrence. Many antioxidant foods and supplements will be helpful, but at present, it is judged that maintaining intracellular calcium homeostasis in the blood to increase NK cell activity that destroys cancer and improving the function of p53 is the only way to save time and be freed from cancer. (ref.: SAC calcium and SAC-SG).

*Ref: Front. Immunol., 07 August 2020,,Sec. Cancer Immunity and Immunotherapy Volume 11 - 2020 https://doi.org/10.3389/fimmu.2020.01280

3. Case Study

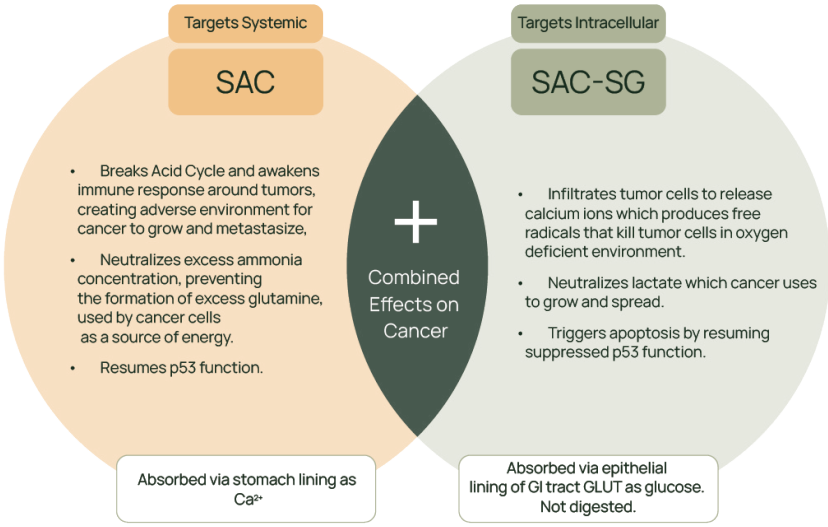


Figure 92. SAC + SAC-SG Combination Cancer Therapy

3a. Mr. Peter Jung (Breast cancer, stage 4, 67 years old (2021), Asian, Male) : Case study of SAC-SG

Diagnosis: Stage 4 Breast Cancer with metastasis in bones and several locations. Diagnosed in February 2021.

Medical History: Mr. Jung experienced significant back pain due to bone metastasis, making it difficult for him to get out of bed.

Pre-Trial Treatments:

- Underwent a Radical Mastectomy, with the removal of 600 grams of tumor/flesh.
- Received chemotherapy and radiotherapy from 2020 to the present, including a second round of chemotherapy.

SAC+SAC-SG Treatment (Parallel with Chemo):
SAC-SG + SAC has been administered three times a day since October 5, 2022.

By October 11, 2022, the back pain had been significantly reduced to 10-20%.

Tumor marker CA15-3 levels:

- 470 (September 20, 2022) – Before the treatment.
- 294 (October 13, 2022) – After 8 days of the treatment.
- 205 (November 28, 2022) – After 54 days of the treatment.
- 135 (January 10, 2023) – After 95 days of the treatment.
- 125 (January 25, 2023) – After 110 days of the treatment.
- 75 (February 22, 2023) – After 138 days of the treatment.
- 35 (August 30, 2023) – After 302 days of the treatment.

3b. Ms. Reidel (Rectal cancer, stage 4, 80 years old (2023), Caucasian, Female) : Case study of SAC-SG

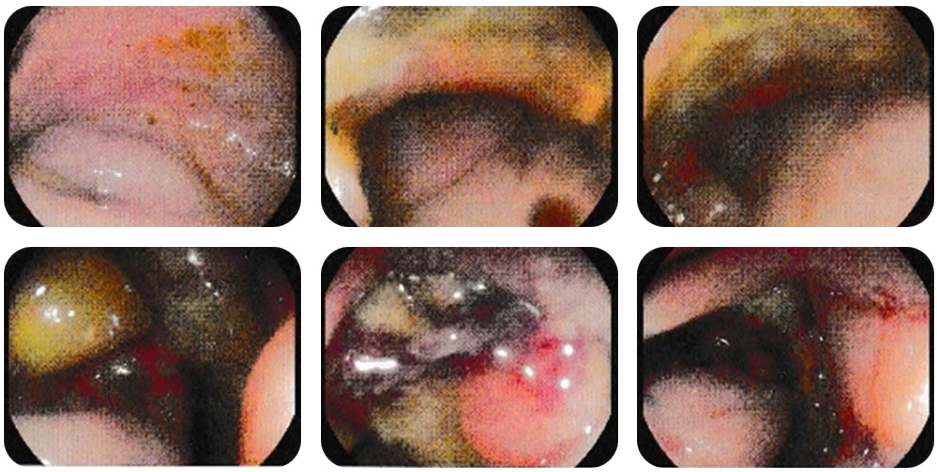


Figure 93. Rectal Cancer Stage 4 CT scans before SAC+ SAC-SG Treatment (May. 2023)

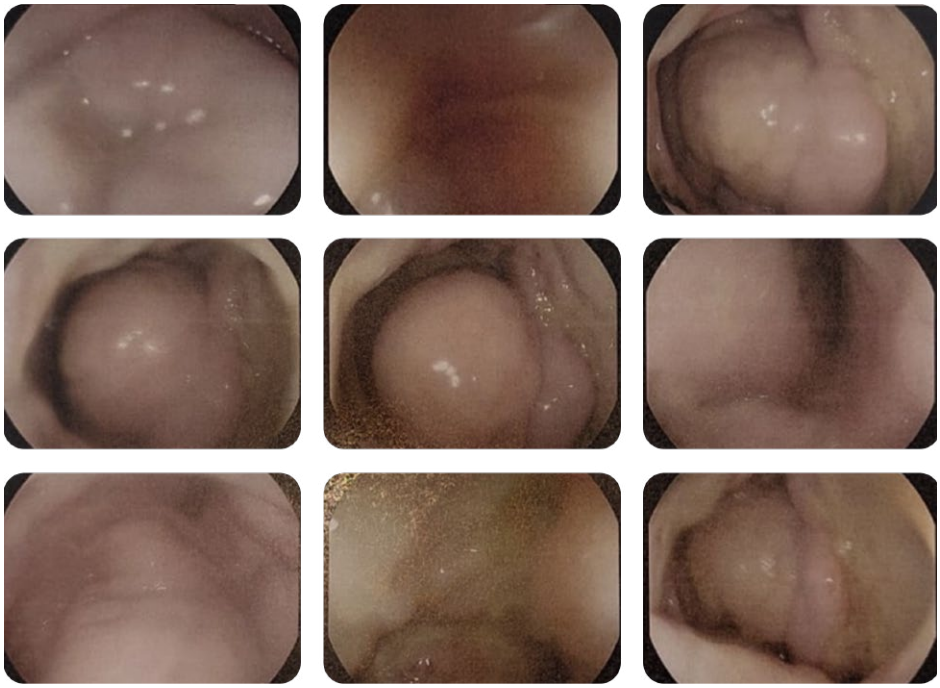


Figure 94. Rectal Cancer Stage 4 CT scans after SAC+ SAC-SG Treatment (September. 2023)

Date	SAC Reference	Cancer Marker	Note
JULY 11th			• Lost weights last few months. (but not skinny) • Appetite is okay. • Potential rectovaginal fistula
July 15th (1 day of SAC)	250mL SAC-SG: 3 times/ day 250mL SAC: 3 times/day for 16 days		1st day of SAC +SAC-SG
July 17th			• Normal Bowl movement • A bit weaker than previous weeks (difficult waking, hard to getting up from chair) but good mood and stable. • Taking antibiotic together due to urinary infection

Date	SAC Reference	Cancer Marker	Note
July 19th			• Completion of 26 times of radiotherapies
July 20th			• Severe hip pain (Significant decrease in her mobility last 3-4 days)
July 24th (9 days of SAC)		**CA19-9**: 44.3 U/mL (Normal range Less than 37 U/mL) **CEA**: 122 ng/mL (Normal range: Less than 5 ng/ml)	
July 26th			• Stable and good mood/regular bowl movement • Joints, shoulder and hip pain
Aug 4th (20 days of SAC)	July 24th order 250mL SAC-SG 250mL SAC 1-2 times/day		• Appetite/bowl movement -normal • Hip pain slightly better • Heart check up (great condition)
Aug 14th (30 days of SAC) • Chemo postponed • Early Sept (surgery discussion)		**CA19-9**: 36.37 U/mL (Normal range Less than 37 U/mL) **CEA**: 70.7 ng/mL (Normal range: Less than 5 ng/ml) • Urinary infection (protentional Fistula) • Overall condition stable • Very good appetite/ normal bowl movement • Still joint pain	
Sept 15th (62 days for SAC)	Sept 8th order 250mL SAC-SG 250mL SAC 1-2 times/day	**CA 19-9**: 18.9/mL **CEA**: 27.9ng/mL • Tumor size same as before. • Abnormal bowel movement - last two weeks (abnormal bowel movement/ 2-3days constipation and diarrhea for 1 week) • Mood is okay • No pain	

Table 15. Rectal Cancer Stage 4 Patient, SAC+ SAC-SG Treatment Progress (2023)

X. Cancers and Gut Microbiome Biofilm

1. Cancer is cellular, so aren't microbes involved?

An estimated 30% of all cancers are linked to microbial agents. Microbes are intimately involved in many cancers. They influence the risk for developing cancer, immune responses that affect both cancer and tumor control, disease progression, and response to treatment. Bio-Sacal, as a prebiotic, focuses on the microbiome, which is something we've been doing since 2011.

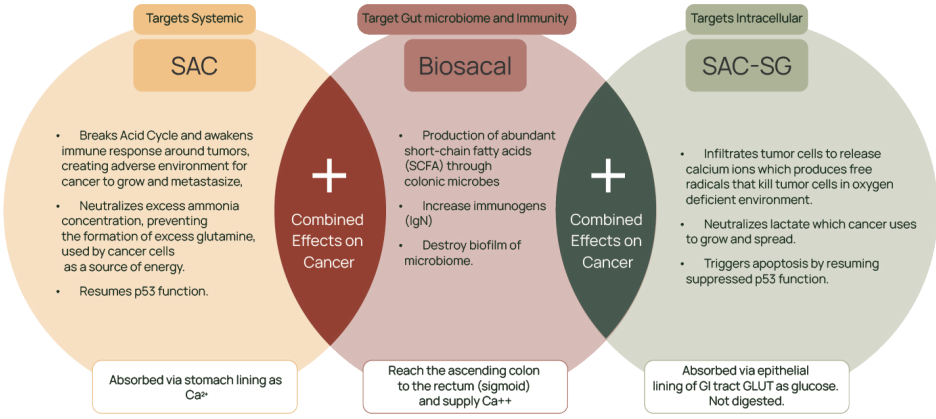


Figure 95. 3D SAC Therapy for Cancer

The number of human cells is approximately 35 to 40 trillion (approximately 23,000 genes per cell) for adults, and the number of microorganisms in the body is approximately 38 to 42 trillion (3 million genes). Humans have 8 to 17 carbohydrate-decomposing enzymes, but intestinal microorganisms have approximately 15,000 to 20,000 carbohydrate-decomposing enzymes. Most of these microorganisms live in the intestines, and there are approximately 800 to 1200 species, depending on eating habits, climate, and race.

We know through many microorganism studies that these microorganisms have a good or bad effect on the immune system depending on the chemicals they secrete, and 70 to 80% of immune function comes

from the large intestine. Therefore, managing intestinal microorganisms through diet is most important.

Most of these exist in the large intestine, but they are distributed to all organs from the skin and mouth. They exist not only in acute and chronic inflammation and tumors but even in brain cells. P. gingivalis, which is found in the brains of dementia patients, is shocking us. Therefore, the production of abundant short-chain fatty acids (SCFA) through colonic microbes is very important for the formation of healthy immunogens (IgN). In other words, if the SCFA level is low, the probability of inducing various cancers, ulcers, dementia, autism, depression, allergies, rare diseases, and mental illness increases. In particular, there seem to be few scholars who oppose the claim that colon cancer and rectal cancer are largely caused by colonic bacteria.

2. Microbial involvement in leading causes of death in the US (Centers for Disease Control 2017)

Asterisks (*) denote the evaluation of the strength of microbial involvement:

- Heart disease: 633,842 *** (an estimated 30% of all cancers are linked to microbial agents)
- Cancer: 595,930 ** (an estimated 20% of all cancers are linked to microbial agents)
- Chronic lower respiratory disease: 155,041 **
- Accidents: 146,571
- Stroke (cerebrovascular disease): 140,323 **
- Alzheimer's disease: 110,561 ***
- Diabetes: 79,535 ***
- Influenza and pneumonia: 57,062 ***
- Nephritis, nephrotic syndrome, and nephrosis: 49,959 **
- Suicide: 44,193 ** (microbiota are closely linked to depression, which is a major cause of suicide)

There are many probiotic products on the market that promote the growth of beneficial bacteria in the gut, but few probiotic products reach the ascending colon to the rectum and are effective. There is a very close

relationship between the level of ionic calcium in the gut and the bacteria that secrete SCFAs. It has been successful in increasing beneficial bacteria by reaching the ascending, transverse, descending, and rectum (sigmoid).

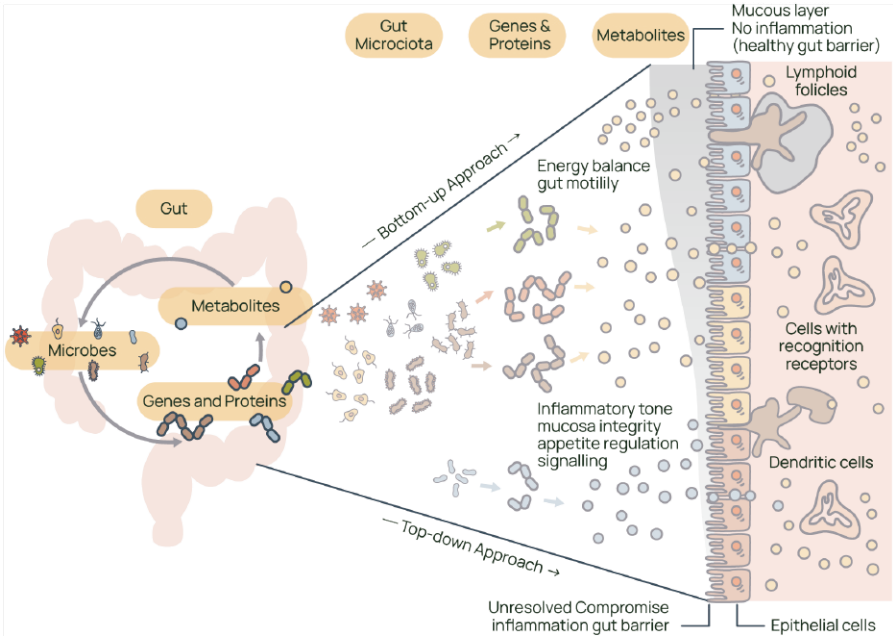


Figure 96. Moya & Ferrer / Trends in Microbiology

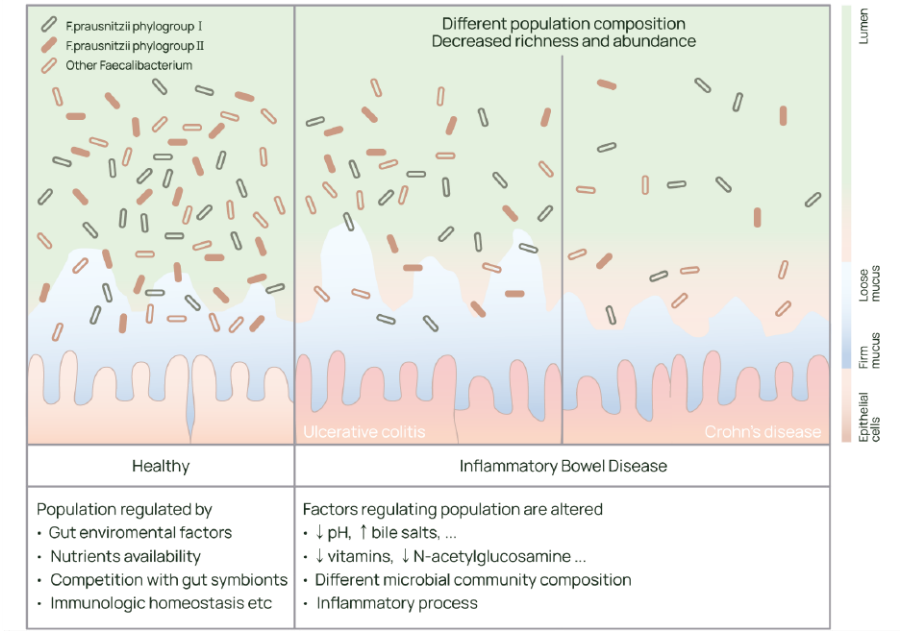


Figure 97. Gut Microbiota in Health and Disease

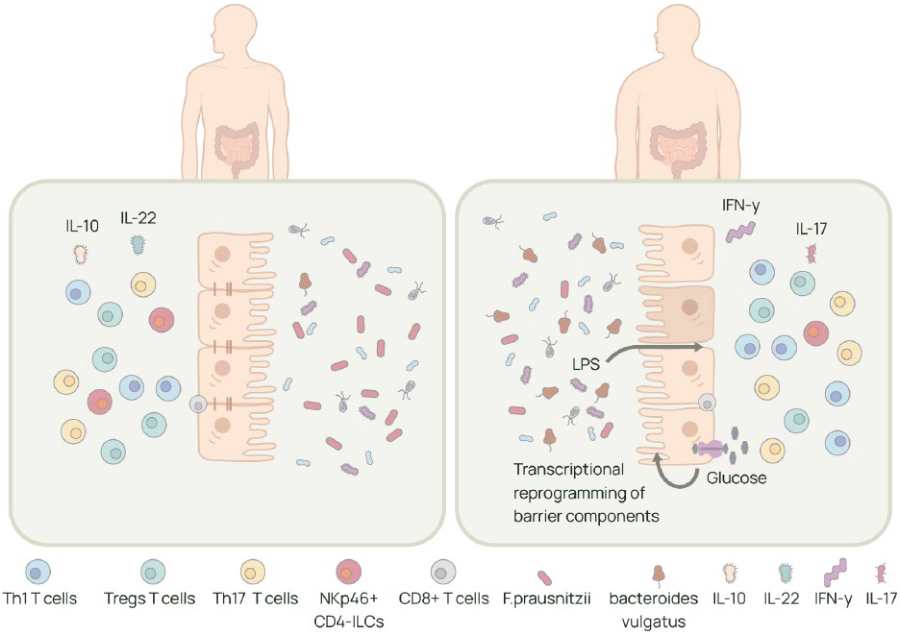


Figure 98. Possible Benefits of Faecalibacterium prausnitzii for Obesity-Associated Gut Disorders

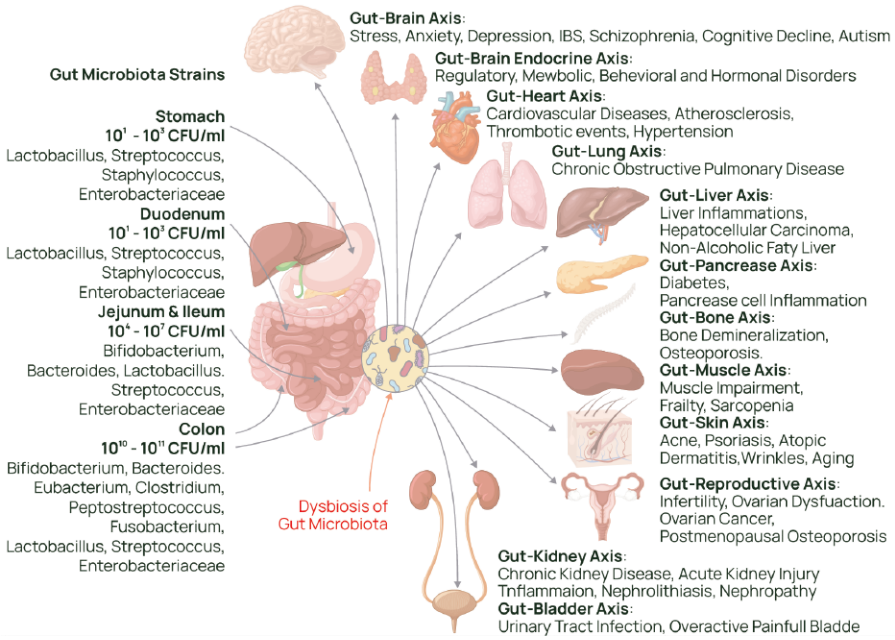


Figure 99. Effects of Gut Microbiota on Various Body Axes

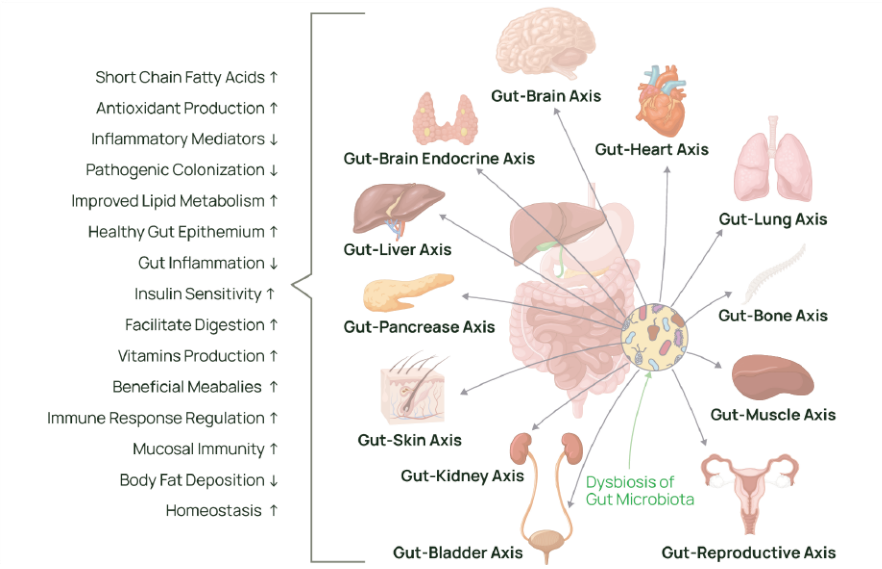


Figure 100. Gut Microbiota Strains and Their Influence on Different Body Axes

3. Mapping Human Microbiome Drug Metabolism by Gut Bacteria and Their Genes

Individuals vary widely in their responses to medicinal drugs, which can be dangerous and expensive owing to treatment delays and adverse effects. Although increasing evidence implicates the gut microbiome in this variability, the molecular mechanisms involved remain largely unknown. Here we show by measuring the ability of human gut bacteria from diverse clades to metabolize orally administered drugs that many drugs are chemically modified by microorganisms. We combined high-throughput genetic analyses with mass spectrometry to systematically identify microbial gene products that metabolize drugs. These microbiome-encoded enzymes can directly and substantially affect intestinal and systemic drug metabolism in mice and can explain the drug-metabolizing activities of human gut bacteria and communities based on their genomic contents. These causal links between the gene content and metabolic activities of the microbiota connect interpersonal variability in microbiomes to interpersonal differences in drug metabolism, which has implications for medical therapy and drug development across multiple disease indications.

In particular, long-term use of antibiotics, anticancer drugs, etc., has a very negative impact on the gut microbiome, leading to a loss of immunity and making recovery from disease very slow or impossible.



Figure 101. Bristol stool Chart

4. SAC Calcium and Biofilm of Microbiome





Dairies have used calcium to manage sewer pipes clogged due to biofilm formation of microorganisms in dairy wastewater. Biofilm formation in dairy wastewater system irrigation pipes can reduce treatment capacity, increasing maintenance and cleaning costs. Understanding the effect of different components in the wastewater on the growth and yield of bacteria present could help prevent excessive build-up of biofilms. This study investigated in aerobic and anaerobic conditions the effect of calcium, sodium, and magnesium concentrations on growth rates, yields, and saturation constants of four known biofilm-forming bacteria associated with the blockage of an irrigation system.

The ions tested (Ca++, Mg++, Na+) varied growth rates, with biofilm growth in the presence of calcium being significantly slower ($P < 0.05$) than planktonic growth in sodium. Along with the slower growth, the addition of Ca++ (up to 0.1 M) increased biofilm formation, while addition over 0.5 M prevented biofilm formation. Knowing the nutritional requirements of the bacteria and the effects of the ions will be useful in predicting the growth development and strategies in controlling biofilm formation in dairy wastewater.

The effect of calcium on biofilm formation in dairy wastewater
by School of Food and Nutrition, Massey University, New Zealand, 2018.
Water Practice & Technology Vol 13 No 2.

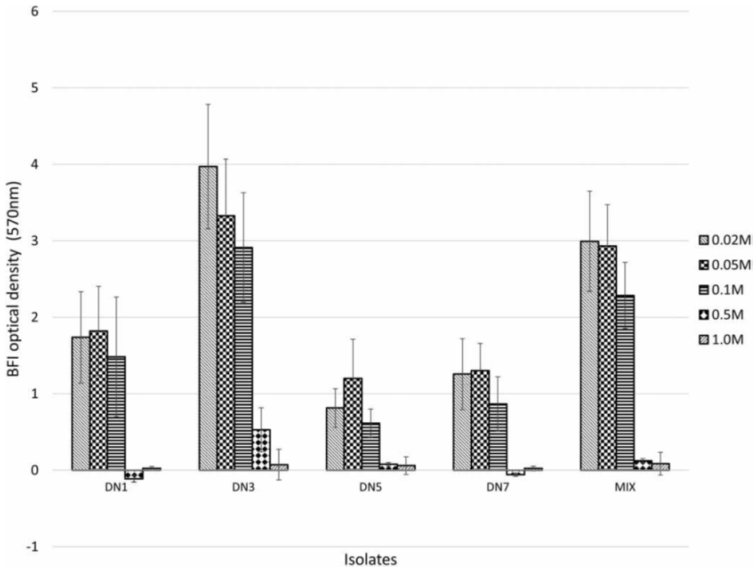


Figure 102. BFI mixed culture results in the presence of Ca++ (570 nm). Error bars represent 95% confidence intervals on nine measurements. Calcium is the independent variable. The addition of 0.5 M or greater completely inhibited biofilm formation.

Bacteria protect themselves from the external environment by creating a defense barrier called biofilm while performing quorum sensing. The same is true for the large intestine of the human body. When bad bacteria gather and form biofilm due to an imbalance in the diet, toxic substances are secreted, and when these act on mucosal cells, they will naturally harm intestinal health.

Many types of products are being released to balance the gut microbiome. Starting with probiotics, prebiotics, and products, recently synbiotics, postbiotics, and parabiotics have also been produced and sold. However, although some products are partially effective, the effect is still insufficient.

For this reason, if SAC calcium is coated with fiber to prevent absorption in the duodenum and small intestine and sent down to the rectum, SAC calcium contained in Bio-Sacal reaches the large intestine and rectum and supplies ionic calcium to beneficial bacteria (Akkermansia muciniphila,

Bifidobacteria, Christensenellaceae, Faecalibacterium, etc.) that secrete SCFA in the intestine to activate their metabolism, thereby increasing the abundance of beneficial bacteria and eliminating harmful bacteria.

Through this SCFA (short chain fatty acid), it not only normalizes the immune function but also relatively reduces the secretion of harmful substances, strengthens the intestinal mucosa and intestinal muscles, eliminates constipation, and greatly helps colon health. The results of this study led to the launch of Bio-Sacal, a prebiotic, in Feb. 2023.

Bio-Sacal is a prebiotic that supplies calcium ions to all parts of the intestines by coating calcium with fiber through a special process so that it reaches the rectum, thereby maintaining the balance of intestinal microflora and increasing the production of short-chain fatty acids by bacteria such as Akkermansia, thereby enhancing immunity.

5. Case Study: Age 60 Male, NY, USA 2021 with Rectal Cancer Stage 3

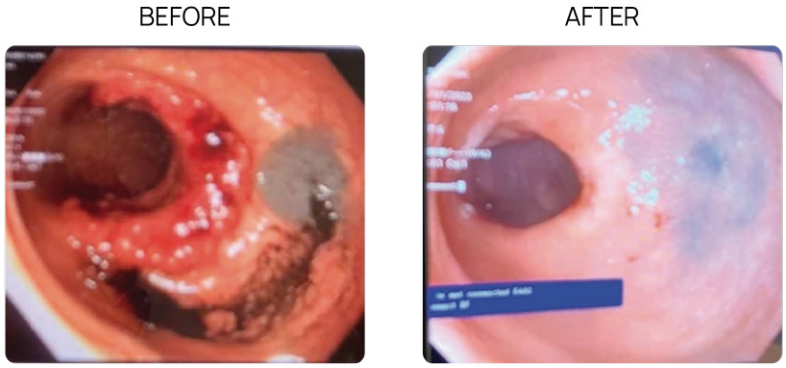


Figure 103.

Rectal Cancer Stage 3 CT scans before and after SAC calcium, SAC-SG, Bio-Sacal protocol used

Medical History:

- (9/28/22) CT chest showed no evidence of metastatic disease.
- (10/3/22) Lower EUS showed a rectal mass consistent with adenocarcinoma, with a few abnormal lymph nodes in the perirectal region, uT3uN1.
- (10/4/22) MRI pelvis showed a T2N2 high/mid rectal tumor.
- (10/25/22) Started concurrent chemoradiation with capecitabine; completed on 12/2/22.
- (12/16/22) s/p mediport placement; started CAPOX; completed cycle 4 on 2/17/23.
- (2/10/23) CT C/A/P showed improved mild wall thickening in the rectum and gallstones; no evidence of metastatic disease.

Diagnosis: Rectal Cancer Stage 3, diagnosed in February 2021. Patient had a colonoscopy on September 15, 2022, which showed an ulcerated and fungating mass 8cm from the anus, consistent with moderately differentiated adenocarcinoma. Mismatch repair (MMR) status is intact.

Pre/Concurrent SAC Treatments:

- Radiation treatment ended on December 2, 2022.
- SAC+ SAC-SG therapy started on December 14, 2022.
- The first round of chemotherapy began on December 16, 2022.
- The second round of chemotherapy commenced on January 6, 2023.
- The fourth round of chemotherapy concluded in early March.

3D-SAC Therapy for Cancer: SAC-SG + SAC + Bio-Sacal was administered three times a day since December 14, 2022. The first two rounds of chemotherapy posed no difficulty. The third and fourth rounds of chemotherapy posed slight difficulty.

On February 10, 2023, three CT scans were performed, and the results showed that the affected rectum had responded well to treatment. On March 1, the surgeon in charge of the surgery performed a precision endoscopy and found no visible tumor at the site. The opinions of the doctors were divided on whether surgery should still be performed or not. On March 14, 2023, an MRI was performed to make the final decision.

Remarkably, all signs of cancer had disappeared. Consequently, any plans for surgery and further chemotherapy were canceled on March 17, 2023, and scheduled check-ups were set every 3-6 months.

XI. AIC, Cancer and the Biological Terrain

- Integrative Cancer Therapy -

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1. Introduction

According to the World Health Organization (2022) and GLOBOCAN (Sung et al. 2021), with an estimated 19.3 million cases and nearly 10 million deaths in 2020, cancer has outpaced cardiovascular disease as the leading cause of death worldwide. These numbers are expected to grow to 29.5 million new cases (+53 %) and 16.4 million deaths (+66 %) by the year 2040 (Ferlay et al. 2019). Especially prevalent in high-income countries, its increasing incidence along with that of other chronic degenerative diseases can be traced to changes in dietary habits, lifestyles and the environment. According to the Canadian Cancer Society (2021), the cancer burden in Canada was estimated at 229,200 cases and 84,600 deaths, while the American Cancer Society (2021) estimated 1.9 million new cancer cases and over 608,570 deaths in the United States in 2021. As the COVID-19 pandemic overtook the world, it created negatively poised uncertainties that led to under-reporting of disease incidence and delays in time-sensitive medical care. It would not be surprising if this is followed by inevitable increases in advanced-stage cancer diagnoses and a corresponding uptick in mortality rates (Dinmohamed et al. 2020, Maringe et al. 2020, Sharpless 2020, Sung et al. 2021).

Metastatic cancer accounts for over 90 % of cancer-related deaths (Steeg 2006). A closer scrutiny of the 5-year survival data for metastatic cancer patients in the United States NCI-SEER database shows no significant improvement since 1996, as illustrated for five commonly occurring cancers in Figure 90 (Ries et al. 2005, Howlander et al. 2017,

Howlander et al. 2021).

Figure 91 demonstrates the substantial drop in 5-year survival rates as cancer progresses from localized to metastasized (distant) forms. This data clearly underlines the need for eliminating misconceptions about the nature of the disease and a paradigm shift on how to treat it.

Current cancer treatments include surgical operation, chemotherapy, radiation therapy, immunotherapy, targeted therapy, hormone therapy, stem cell transplant and precision medicine. Even though there are a lot of cancer treatment options, none of them has shown significant effects for conquering cancer. Data shows that only four percent of tumor cancer patients survived through chemotherapy (Pignon et al. 2000). In another study, it was shown that the condition of lung tumor patients remains poor, despite the use of radiation therapy (Wisnivesky et al. 2010). The reason is because the current cancer treatment options only focus on decreasing the number of pre-existing tumor cells by interfering with cellular mechanisms such as tumor cell division and angiogenesis (sprouting of new blood vessels that feed the tumors). However, to move closer to a real solution, we must understand the underlying mechanism of proliferation of cancer cells in the human body and address the larger issues of bioregulation that lie at the root of the disease.

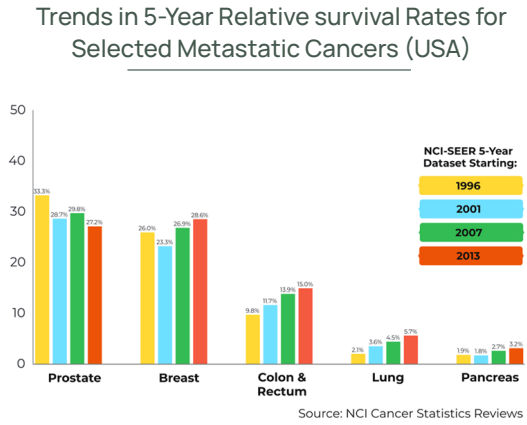


Figure 104. 5-Year relative survival rates for selected metastatic cancers in the United States since 1996 reveal mostly increasing trends (Ries et al. 2005, Howlander et al. 2017, Howlander et al. 2021).

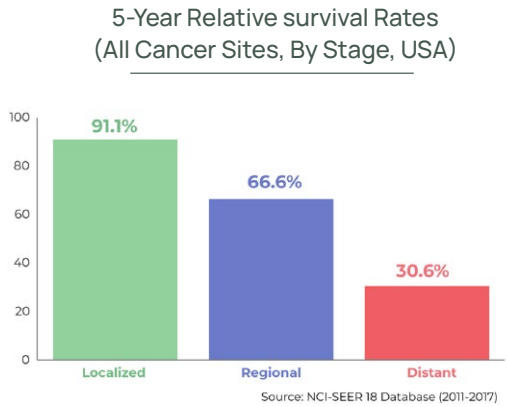


Figure 105. 5-Year relative survival rates for all cancer sites in the United States, classified by stage (Howlander et al. 2021).

Mutated cells will keep duplicating if the overall body metabolism and functions of the cell are not restored to a healthy state. It is crucial to develop new cancer treatments that restore the body's autoregulatory potential and regenerate cellular function, which eventually prevents the subsequent proliferation of tumor cells.

In this regard, AIC Therapy can be utilized to regenerate malfunctioning mitochondria and endoplasmic reticulum (ER), which are instrumental in preventing possible protein mutations such as those of the tp53 gene. By stimulating hormone secretion that plays a critical role in calcium homeostasis and fluctuating influx and efflux of calcium ions in the mitochondrial intercellular membrane, we can finally trigger the apoptosis (programmed cell death) of cancer cells by efficiently releasing apoptosomes such as cytochrome c.

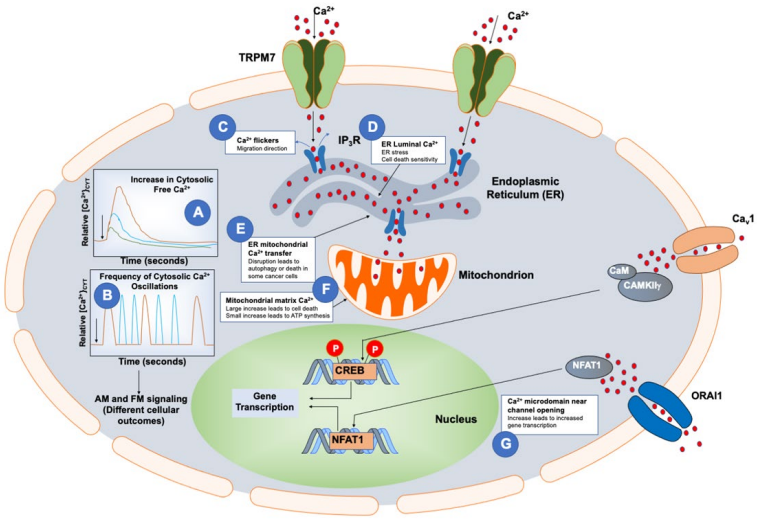


Figure 106. Ca2+ signal diversity in a cell that can contribute to processes relevant to cancer progression. (A) The amplitude modulation (AM) of cytosolic free Ca2+ concentration which can control cell fate; (B) Frequency of free cytosolic Ca2+ concentration oscillations (frequency modulation (FM)) which may control gene transcription and, hence, different cellular outcomes and expression remodeling; (C) Localized and transient increase at the edge of some cells (Ca2+ flickers) which control directional migration of Ca2+ ions; (D) Free Ca2+ ion levels within the endoplasmic reticulum (ER) that may influence cell stress and the Ca2+ ion concentration-dependent sensitivity to apoptotic stimuli; (E) The confined transfer of Ca2+ from the ER to the mitochondria which, when reduced, can induce autophagy or death in some cancer cells; (F) Levels of Ca2+ within the mitochondrial matrix where modest changes can lead to ATP synthesis, but sustained high levels can promote cell death; (G) Localized very high levels of Ca2+ (known as Ca2+ microdomains), achieved upon the opening of plasmalemmal Ca2+ channels, can specifically regulate gene transcription pathways. Adapted from: (Monteith et al. 2017).

2. Ca^{2+} Concentration Modulation in the treatment of cancer

Concentration modulation of calcium ions plays a critical role in cellular metabolism. In that context, AIC Therapy provides a one-of-a-kind, practical and affordable method of leveraging Ca^{2+} modulation in the treatment of cancer, amenable to its use in integrative therapeutic protocols (Figure 94).

In this section, we will cover some critical areas used by cancer to hijack cellular metabolism and how AIC Therapy can be useful in restoring bioregulatory balance.

2a. AIC Neutralizes Lactic Acid Production in Cancer Cells

Healthy cells typically produce energy through a sequential combination

of glycolysis (in the cytosol) and oxidative phosphorylation (in the mitochondria). This results in the formation of 36 ATP per mole of glucose. When oxygen is scarce, healthy cells are restricted to anaerobic glycolysis which nets a total of only 2 ATP per mole of glucose. Cancer cells adapt their metabolism to bypass the oxidative phosphorylation step, even in the presence of sufficient oxygen (Figure 93). They metabolize glucose via aerobic glycolysis followed by fermentation of the product pyruvate to lactate a combined process that does not use oxygen during catabolism and nets 2 ATP per mole of glucose (Vander Heiden et al. 2009). Cancer cells compensate for the low ATP turnover by genetically adapting the cell to increased nutrient intake at the expense of the surrounding healthy cells. They also generate acidic products (lactic acid) whose accumulation creates a favorable tumor microenvironment for the proliferation of cancer cells. Furthermore, in this environment, they go through biosynthesis pathways to produce lipids for pro-tumorigenic lipid signaling, such as palmitate (Louie et al. 2013, Carta et al. 2017).

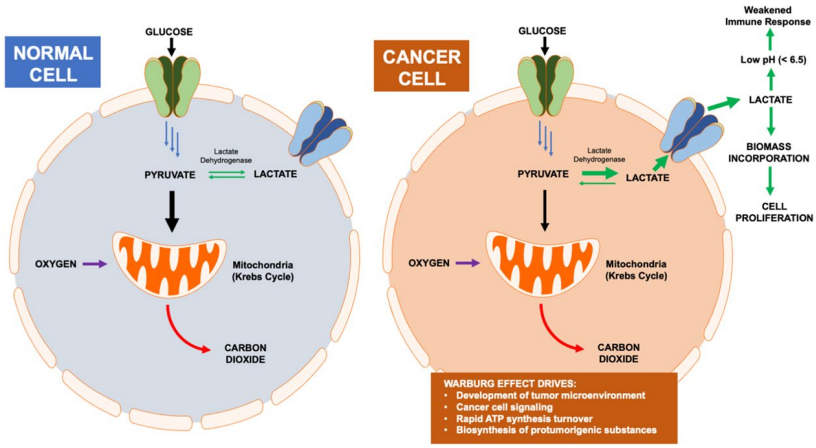


Figure 107. (LEFT) Normal cells drive most of their energy production (ATP) through the glycolytic formation of pyruvate which is metabolized to carbon dioxide in the mitochondria via the Krebs Cycle and oxidative phosphorylation. (RIGHT) Cancer cells drive the formed pyruvate through a fermentative step to generate lactate, largely bypassing the mitochondrial metabolic steps. The release of lactate is critical, since it alters the extracellular matrix and drives key reactions that promote cancer cell proliferation. This process was first outlined in the 1920s by Otto Warburg, for which he was awarded the Nobel Prize in 1931. Adapted from: (Ferreira 2010, Baltazar et al. 2020).

Researchers have postulated that the excessive amount of lactic acid production via cancer cells would activate the secretion of proteases; which causes extracellular degradation, leading to metastasis of cancer cells (Han et al. 2013).

Moreover, the enormous secretion of lactic acid would increase the synthesis of HIF-1 proteins, which are actively involved in the proliferation of cancer cells by invading and metastasizing the healthy cells (Han et al. 2013). Therefore, it is crucial to neutralize the acidic environment in the cell.

The use of AIC solution can neutralize acidic conditions in the tumor microenvironment because calcium ions can react with lactic acid to form the neutral salt, calcium lactate (Figure 95).

The acidic environment interferes with the activity of natural killer cells, T- lymphocytes and NF-κB cells. By forming calcium lactate, AIC therapy reduces the viability of cancer cells by restoring the tumor microenvironment to a more normal state.

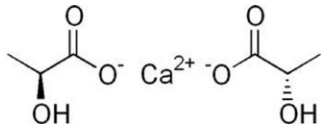


Figure 108. Structure of calcium lactate.

Patients suffering from lactic acidosis showed lack of ionized calcium concentration in the intracellular membrane (Cooper et al. 1992). Gupta argued that cancer cells use aerobic glycolysis which increases degradation of glutamine that "leads to metabolic acidosis with pH of the solid cancers as low as pH 6.0 - 6.5" (Gupta 2016). In other words, lack of calcium ion causes an increase in the presence of lactic acid in the cellular membrane, and therefore, supplementing the calcium concentration at an intracellular level is critical to stabilizing the acidic pH level. Strictly speaking, adequate calcium intake is highly recommended for those who are suffering from cancer.

Unlike regular calcium supplements sold in the market, AIC does not contain severe adverse effects such as renal failure. Moreover, with its

unique structure and efficient absorption method, it can efficiently trigger calcium homeostasis in the body. Also, as it effectively triggers the calcium signaling with the tiny amount of calcium carbonate solution, it can be highly useful to neutralize the cellular pH level as it is ingested.

To investigate the efficacy of AIC in neutralizing cellular pH level, Korean National Sports University held an experiment in 2014 on a group of 20 professional Korean swimmers. They examined whether AIC would decrease the amount of lactic acid and glucose production in the cells following hard physical training. The result was noticeable because the average lactic acid level for the swimmers was notably decreased after orally ingesting 5 mL of AIC twice a day for two weeks . This experiment provides evidence of the ability of AIC to reverse acidosis in the tumor microenvironment, making it harder for the cancer cells to proliferate and tumor to grow. Moreover, it was also observed that the swimmers' personal performance record improved as well after taking AIC . This could be attributed to AIC's ability to regenerate mitochondrial function, which results in higher ATP production.

2b. AIC Reduces Ammonia Concentration in Cancer Cells

Cancer cells require a lot of nutrients to form their building blocks. They uptake a massive amount of glucose as the primary source of energy that activates their cellular activity. However, cancer cells also utilize other substrates that lead to other metabolic wastes as well during catabolism. One such waste product is ammonia (NH_3), which is known to be generated as a waste product in cellular metabolism (Figure 96).

However, as detailed by scientists at Harvard University, ammonia is not merely a toxic waste product of cancer metabolism (Spinelli et al. 2017). Instead, it is used as a resource for the generation of amino acid building blocks, such as glutamine, that support building of the tumor biomass and metastasis. As far back as 1955, Eagle postulated that glutamine is the most utilized nutrient for tumor cells which is why it is key to repress glutamine production to inhibit cancer cell proliferation (Eagle 1955).

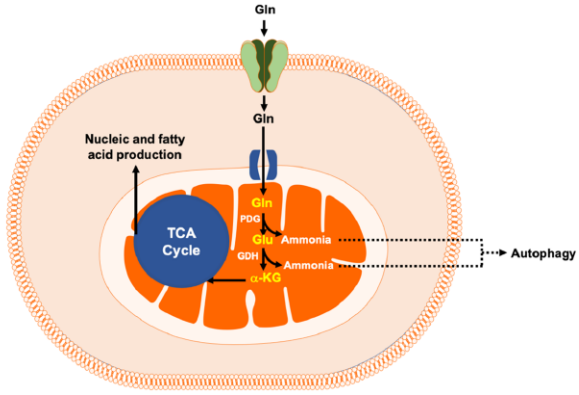


Figure 109. Ammonia as a stress signal in cancer. Ammonia has long been seen as a toxic by-product of glutamine metabolism in mitochondria. (Gln = Glutamine; Glu = glutamic acid; TCA cycle = tricarboxylic acid cycle; PDG = phosphate-dependent glutaminase pathway; GDH = glutamate dehydrogenase pathway) Adapted from: (Edelson 2010).

Furthermore, when there is too much ammonia concentration in the body, the urea cycle is activated in the liver to convert toxic ammonia into uric acid (Ali et al. 2014). However, as cancer cells produce an excessive amount of ammonia, the excretory system does not work correctly, which ultimately causes the body to become highly basic (Spinelli et al. 2017). As cancer cells multiply, a significant concentration of ammonia remains in the cardiovascular system and extracellular mitochondrial matrix (Tarantino et al. 2009), which can cause the dysfunction of mitochondria and alteration in protein synthesis (Niknahad et al. 2017).

According to Spinelli et al. (2017), tumor cells use glutamine to protect themselves from the attack of natural killer cells such as T-lymphocytes and NF-kB Cells. Researchers found that, as they repress the expression of TXNIP protein, cancer cells can invade healthy cells without interruption (Morrison et al. 2014). In an experiment with a mouse model of thyroid cancer, they found that TXNIP expression can weaken the growth of the cancer cells. Moreover, it was also found that TXNIP expression is remarkably activated in the presence of glucose, but significantly suppressed in the presence of both glucose and glutamine (Kaadige et al. 2009). Regulating the ammonia levels should substantially reduce the biosynthesis of glutamine and thereby allow the activation of TXNIP.

So, how can AIC Therapy help? As described in 1970 (Dickman et al.

1970), calcium ions and ammonia undergo a reversible reaction in the gas phase that results in the formation of a calcium ammoniate complex, as shown in the reaction below:

$$Ca^{2+}(g) + 6NH_3(g) \rightleftharpoons Ca(NH_3)^{2+}_6(g)$$

In solution, calcium ions react with aqueous ammonia to form a similar water-soluble complex. Based on the Le Chatelier's Principle, the increase in serum calcium ion concentration by the introduction of AIC can act as a sponge to remove excess ammonia accumulated in the cells and extracellular matrix, and thereby reduce its impact on the growth and division of cancer cells. The expected reactions could be as follows:

2c. AIC Triggers p53 Modification and Apoptosis

The tumor suppressor gene, p53 is known to inhibit cancer cell proliferation and regulate the cell cycle by arresting cellular growth. However, when the calcium level is imbalanced in the body, mitochondria do not work correctly, especially when there is excitotoxicity due to the massive influx of calcium ions into the mitochondrial matrix (Stanika et al. 2009). This destroys the mitochondria, which ultimately causes the mutation in the synthesis of the protein associated with the p53 gene.

The mutation of the p53 protein to the tp53 form (tp = tumor protein) promotes the unchecked proliferation of cancer cells. As p53 is mutated, apoptosis does not occur correctly; therefore, it is critical to maintain calcium homeostasis to prevent mitochondrial dysfunction. Conversely, a disruption in calcium homeostasis can result in mitochondrial dysfunction, leading to cancer cell proliferation.

In addition to the relationship between calcium excitotoxicity and mitochondrial dysfunction (Stanika et al. 2009), we also know that the production of reactive oxygen species (ROS) due to the malfunction of mitochondria causes misfolding of p53 proteins (Murphy 2013). Additionally, imbalance in pH also leads the mitochondrial dysfunction (Hughes and Gottschling 2012). As mitochondrial dysfunction causes the p53 mutation, it is critical to regulate mitochondrial function. As the greater abundance of ionic calcium leads to the regulatory influx and efflux of calcium

ions through the membrane ion channels, calcium signaling is activated which helps restore mitochondrial function.

The p53 gene plays a role in stimulating calcium signaling in order to activate mitochondrial activity to control the expression of oncogenes (Giorgi et al. 2015). However, as p53 is mutated, calcium signals will not work correctly anymore, which ultimately causes the mass production of ROS, which, in turn, affects the development of cancer cells (Macip et al. 2003). Moreover, it was argued that calcium signaling should regularly oscillate since the imbalance of calcium influx and efflux in the mitochondrial matrix can cause tumor formation but, when properly regulated, the production of apoptosomes, such as Apaf-1, contributes to the maintenance of calcium homeostasis (Kaur and Sanyal 2011).

By effectively regulating calcium homeostasis in the body, AIC can trigger mitochondrial activity and adversely affect cancer cell growth and division. Moreover, AIC has proven its efficacy in controlling intracellular calcium ion concentrations by regulating pituitary hormones. Therefore, we can assert that AIC can be a treatment option to cure cancer as it neutralizes serum pH level and regulates mitochondrial activity, which prevents p53 mutation and helps to produce apoptosomes.

3. The Role of AIC in Integrative Oncology

AIC Therapy is an excellent example of a treatment that defines a disease not merely by its symptoms and visible manifestations, but by its bioregulatory relationship with the rest of the body. As a result, AIC finds an important place in integrative therapeutic protocols, including at the Hope4Cancer Treatment Centers in Mexico. To illustrate the relevance of this approach, consider what happens when the same virus attacks a healthy vs. an immunocompromised individual the outcomes can be completely different! In contrast, conventional treatments do not understand the whole-body approach and, as a result, miss the forest for the trees.

In this section, we will explain the importance of focusing on the biological terrain for the effective treatment of cancer and contrast that thinking with existing conventional approaches. We will also discuss the 7 Key Principles of Cancer Therapy, the foundation for the treatment protocols developed at the Hope4Cancer Treatment Centers and through

which we have served thousands of patients for over 25 years.

3a. The Biological Terrain and Its Role in Cancer

Whether for cancer or any other chronic disease, an individual's medical belief system influences the nature of his or her therapeutic choices. To make an informed choice, it would be helpful to come to terms with the disease's genesis: like the flu, is it caused by a specific pathogen? An internal flaw in the body's terrain? Or a combination of both? Is it written in the genes we inherit, or is it triggered by environmental factors or both? Do we take a cell-outwards viewpoint by thinking of cancer as initiated by a genetic "flaw" that amplifies into a full-blown disease? Or do we look at the disease system-inwards where influenced by the environment changes in the terrain build up over time to create conditions that favor cancer growth?

The concept of the biological terrain in cancer can be traced back to a paper published in the journal Lancet by Paget in 1889 where he proposed the idea that became famously known as the "seed-and-soil hypothesis"(Paget 1989, Langley and Fidler 2011). Paget wondered how metastasis favored certain "predisposed" organs (e.g., in breast cancer, the liver and bones are common metastatic targets) but spared others. Through animal studies, he also observed how simply injecting an animal with cancer cells resulted in disease in a few, but not all animals. He stated, "When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil"(Paget 1989). Little did he know that over 100 years later, the concept of the tumor microenvironment, invasion and metastasis would mirror his original idea.

3b. The Biological Hallmarks and Physical Traits of Cancer

In two seminal papers published in the years 2000 (Hanahan and Weinberg 2000) and 2011 (Hanahan and Weinberg 2011), Hanahan and Weinberg proposed ten hallmarks of cancer that effectively describe the characteristics that a cancer cell acquires that helps it modify the body's biological terrain to its advantage. The created niche, known as the tumor microenvironment (TME), favors the growth and proliferation of cancer cells

(Figure 97, top). The TME is at the center of how cancer evolves from a single cell to a tumor, and then to life-threatening metastasis that affects both survival and quality of life of the patient. In 2022, Hanahan wrote a third paper in the series where he described four additional hallmarks: unlocking phenotypic plasticity, non-mutational epigenetic reprogramming, senescent cells, and polymorphic microbes (Hanahan 2022). These new hallmarks acknowledge even further the participation of external environmental factors, microbes, and alteration of cells through the aging process as key contributors to the cancer formation and proliferation processes.

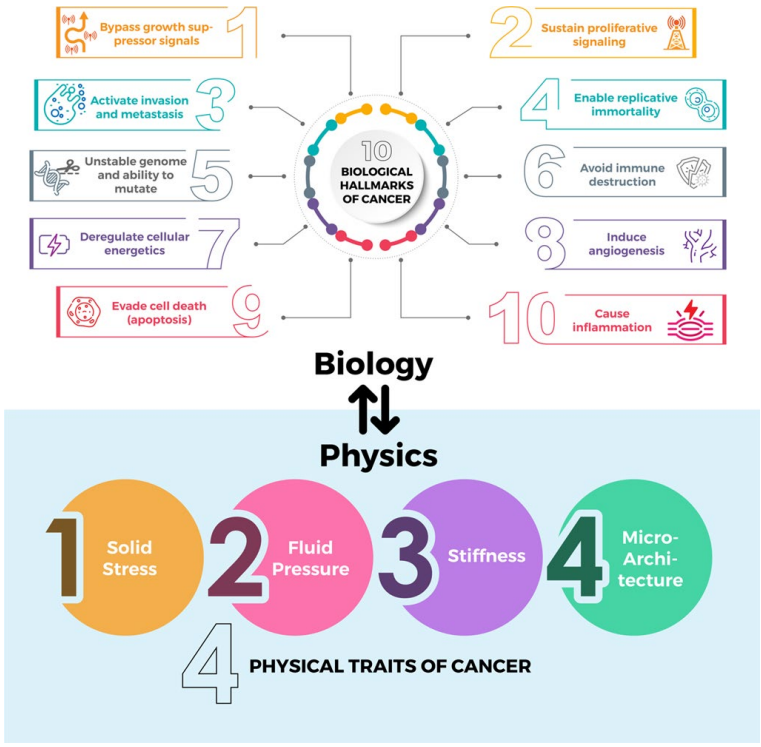


Figure 110. The 10 Biological Hallmarks and the 4 Physical Traits of Cancer: Interaction of biology and physics in the biological terrain (Hanahan and Weinberg 2000, Hanahan and Weinberg 2011, Nia et al. 2020). In addition to these biological hallmarks, Jain et al. have recently summarized four physical traits of tumors in the TME that contribute to their growth and metastasis (Figure 3, bottom) (Nia et al. 2020). These traits show how tumors can change the physical characteristics of their immediate environment in the following ways:

a) By buildup of shear stress that causes physical compression thus restricting the delivery of nutrients and oxygen.
b) By increasing interstitial fluid pressure that causes leaky vessels.
c) By remodeling the extracellular matrix that causes it to become stiff and activates pathways that promote invasion and metastasis.
d) By reorganizing cellular structures.

All of these traits contribute to creating an environment that favors tumor growth and metastasis. At the end of this physical restructuring, the tumor site does not resemble healthy tissue anymore either structurally or functionally (Nia et al. 2020).

These biological hallmarks and physical traits define the intrinsic (within the cell) and extrinsic (outside the cell) factors that cancer cells acquire to become the formidable adversaries we know them to be. They provide the rationale why we identify cancer as being "intelligent," as it hijacks the body's own internal defenses for its own survival. They identify cancer as an organism that will do anything in its power to survive, even if it means adopting a parasite-like existence that subverts the body's natural bioregulatory processes (Smit 2009, Goldman et al. 2015).

3c. The 7 Key Principles of Cancer Therapy™

The 7 Key Principles of Cancer Therapy™ provide an integrative oncology therapeutic blueprint for cancer patients that simultaneously targets cancer cells while healing the body's biological terrain (Jimenez and Chakravarty 2012, Jimenez 2019). This clinical approach perceives healing from cancer as a multidimensional problem, with each principle being a spoke of a seven-sided wheel that must be addressed (Figure 98). As discussed in detail earlier, our improved scientific understanding of the biological terrain's ability to shape the onset, growth and spread of cancer makes the 7 Key Principles approach to cancer therapy not only increasingly relevant academically, but also a method of choice to successfully treat cancer at all stages of disease.

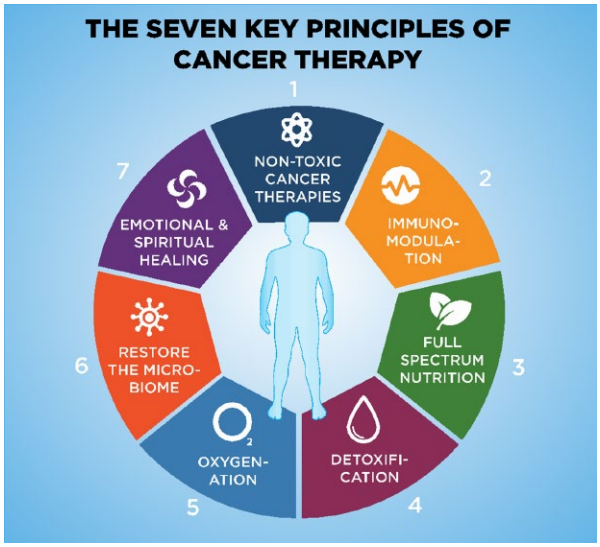


Figure 111. The 7 Key Principles of Cancer TherapyTM (Jimenez 2019).

Principle 1: Non-Toxic Cancer Therapies.

One of the biggest concerns with traditional cancer therapies are the associated toxicities that can lead to short- and long-term side effects, compromised quality of life and shortened lifespans. The treatment-induced immunocompromised state of cancer patients leaves them especially vulnerable to morbidity and mortality, as described in a later section. Non-toxic treatment approaches by definition use treatments that do not add to the toxic burden of the body.

Versatile treatment modalities such as Sono-Photo Dynamic Therapy, for example, use light and sound energy to activate non-toxic sensitizers that preferentially absorb into cancer cells.

Once activated, these sensitizers destroy the cancer cells from within. Mechanistically, Sono-Photo Dynamic Therapy can induce cancer cell apoptosis, activate the immune system, weaken the tumor vasculature, and kill associated pathogens (Figure 98) (Reginato et al. 2014, Jimenez 2019). Similarly, treatment with heat (hyperthermia) is also highly effective in destroying cancer cells and activating the body's immune system (Vaupel 1990, Song et al. 2005).

Hyperthermia is an effective treatment option that is used in both integrative and traditional oncology settings. In the latter, it has been shown to potentiate the effects of chemotherapy and radiotherapy (Datta et al. 2020).

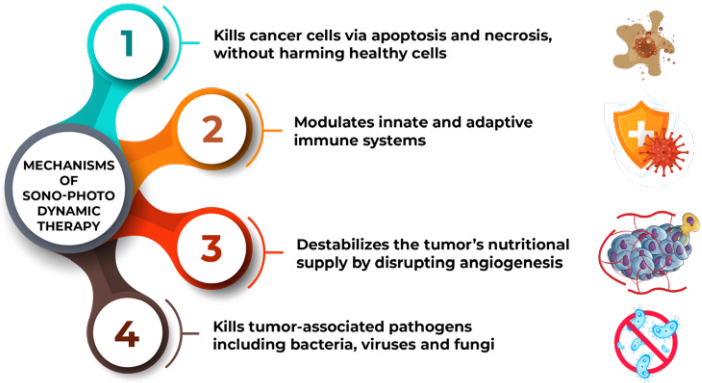


Figure 112. Mechanisms of Sono-Photo Dynamic Therapy (SPDT) (Jimenez 2019).

Principle 2: Immunomodulation.

Innate and adaptive immune system cells work in concert to eliminate a variety of pathogens and other toxins, including cancer cells. In fact, because of this on-going immunosurveillance, immune cells remain on alert to seek and eliminate nascent cancer cells, preventing them from establishing themselves and growing into tumors. The ability of cancer to avoid immune surveillance is one of the first necessary steps in tumor development (Hanahan and Weinberg 2000).

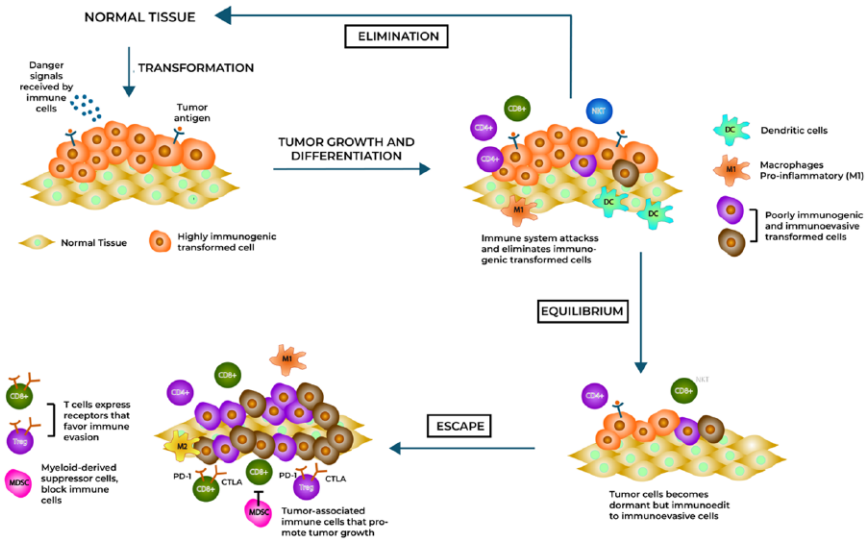


Figure 113. The theory of immunoediting: how cancer bypasses immune surveillance. (Schreiber et al. 2011)

The theory of immunoediting (Figure 99) (Dunn et al. 2004, Dunn et al. 2004, Schreiber et al. 2011) describes the process by which cancer bypasses the immune system. Immunoediting consists of three phases:

a) elimination, where the nascent tumor is completely eliminated by the innate and adaptive arms of the immune system;
b) equilibrium, where immune-resistant cancer cells start to develop in the tumor but are still undetectable;
c) escape, when the cancer cells have escaped the immune system and can now form tumors with cells exhibiting low immunogenicity (susceptibility to the immune system). In fact, the tumor microenvironment is home to many innate and adaptive immune cells that have been altered to ignore or even protect the growing tumor (Gonzalez et al. 2018).

Treatments that modulate the immune system and destabilize the "new normal" in the growing tumor microenvironment are essential components of the 7 Key Principles of Cancer Therapy. These include biological immunotherapy protocols that activate macrophages in the innate immune system and also modulate the population and activity of

dendritic cells and adaptive immune system T-cells.

Principle 3: Full Spectrum Nutrition.

Preventative nutrition can confer a major advantage, reducing cancer risk by 30-50 % (Vineis and Wild 2014). Nutrition also plays a pivotal role in hindering cancer progression and promoting survival. We developed the Garden Food Plan® as a nutritional framework for cancer patients to follow as part of the 7 Key Principles of Cancer Therapy (Figure 98) (Jimenez 2019). Research has shown that while acute nutrient deficiencies are not common in developed countries, suboptimal intake of nutrients is rampant and a likely source of system-wide metabolic imbalances. Research has shown that even a single nutrient deficiency can increase the risk of DNA damage, promote cancer and neurological decay and dysfunction, as well as potentially lead to accelerated aging via disruption of mitochondrial function (Ames and Wakimoto 2002). Once a patient has cancer, they need to compensate for the malnutrition and metabolic derangements induced and accelerated by the disease itself. If a patient is on traditional therapies, they must consider the nutritional deficit caused by toxic treatments (Arends et al. 2017).

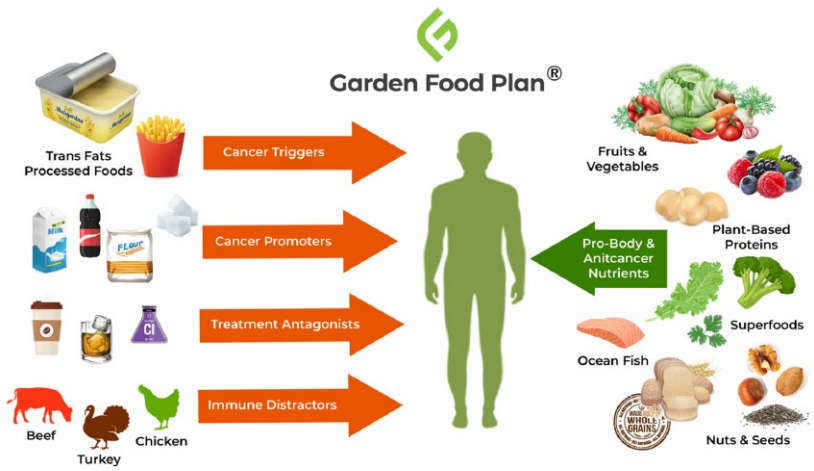


Figure 114. Components of The Garden Food Plan.

Principle 4: Detoxification.

Our bodies have several sophisticated ways of removing physically harmful toxins. Various body systems including the respiratory, immune, digestive, urinary, and integumentary systems (skin) are involved in keeping the toxin load in the body at tolerable levels. However, air, soil and water pollution ensure that we are constantly exposed to many potentially harmful substances every day many of them which have proven carcinogenic potential. Compounded with that, poor daily habits such as unbalanced and suboptimal nutrition, sedentary behavior, sleep deprivation, and lack of physical activity can lead to an accumulation of toxins that exert pressure on our bioregulatory systems (Smit 2009, Thom et al. 2018).

Restoring the body to normalcy from a tumor-favoring disposition requires continual detoxification, which is why it plays a central role in the 7 Key Principles of Cancer Therapy. Some of the techniques used include colon, liver and gallbladder cleanses; infrared saunas; coffee and other types of enemas; drainage of the lymphatic systems; heavy metal removal; and therapeutic breathing (Jimenez 2019). Biological dentistry, another highly valuable detoxification treatment method, focuses on removal of root canals, cavitations, amalgams and the treatment of periodontal diseases. These diseases, if not treated, can remain an important and continuous source of cancer-driving toxins (Levy 2017).

Principle 5: Oxygenation.

The Hallmarks and Traits of cancer provide a foundation for how the tumor microenvironment is formed. One of the consequences of this process is the development of low-oxygen, or hypoxic, conditions. The adaptation of cancer cells to the glycolytic metabolic pathway over oxidative phosphorylation, as described by the Warburg effect (Seyfried 2012, Schwartz et al. 2017) helps them thrive in both normoxic and hypoxic conditions. The ability to survive in hypoxic conditions confers a powerful survival advantage to cancer cells over their healthy counterparts and supports the conversion of beleaguered stromal cells to a cancer phenotype. The hypoxic condition can fuel many pro-cancerous

conditions including: resistance to apoptosis and autophagy; a switch to anaerobic metabolism; inflammation, immunosuppression, and fibrosis; abnormal angiogenesis; resistance to therapy; promotion of cancer stem cell phenotypes; and epithelial-mesenchymal transition that supports invasion and metastasis (Jain 2014).

Reversing hypoxic states is, therefore, crucial in the treatment of cancer at all stages of disease, which is why it finds an important place in the 7 Key Principles of Cancer Therapy. Hyperbaric oxygen therapy and ozone therapy are two important treatments used in integrative oncology to deliver oxygen to the tumor microenvironment, making it less favorable to cancer growth.

Principle 6: Restore the Microbiome.

It is estimated that about 20% of all cancers are directly or indirectly related to infectious agents including viruses, parasites, fungi, and bacteria (Morales-Sanchez and Fuentes-Panana 2014, Vandeven and Nghiem 2014). The role of viruses in oncogenesis is verified by the detection of the viral genome incorporated in the cancer cells (Moore and Chang 2010). While the negative association between pathogen and cancer has been known for many years, the beneficial role of the human microbiota in preventing cancer is only being uncovered in recent years.

A new term to describe an organism is the "holobiont" a combination of the host and the many other species that lives within its immediate environment. The human holobiont is estimated to have a 10:1 ratio of microbial to human cells. The microbiota is seamlessly integrated in the human ecosystem, modulating important parts of the host's physiology such as cellular metabolism and immune function (Fulbright et al. 2017).

Restoring the health of the microbiome is an easily neglected priority even though it can have a profound effect in restoring the biological terrain. Therapeutic considerations include elimination of acute and chronic infections with treatments such as ozone therapy, antimicrobial photodynamic therapy, hyperthermia, and ultraviolet blood irradiation (UVBI) (Jimenez 2019). The use of pre, pro, and postbiotic formulas designed to restore beneficial bacterial colonies in the depleted microbiome is an important way to rebuild healthy microbial populations. Re-establishing

contact with nature and increased physical activity also bring us back in touch with our environment and thereby support the growth of a healthy microbiome.

Principle 7: Emotional and Spiritual Healing.

The 7 Key Principles of Cancer Therapy incorporates BEST (Behavioral Emotional Spiritual Therapy), a comprehensive program that seeks to understand the components of behavior, emotions, and spirituality that may lie at the root of the cancer disease process. In our clinical experience and as substantiated through numerous case studies, we know that releasing associated traumas and biased beliefs plays a major role in healing from the disease (Jimenez 2019). In the past few decades, concepts such as neuro-linguistic programming (NLP), psycho-neuro-endocrino-immunology (PNEI) and psycho-neuro-immunology (PNI) have gained increased traction in the scientific literature, establishing the clear need to define the biological connection between emotional states and the physical body.

Several studies have enumerated the significant relationship between life events, important losses and difficult life situations with the incidence of breast cancer. In a case-control study of 87 breast cancer patients in Finland, Forsén showed that emotional losses and life event scores were correlated with subsequent development of breast cancer (Forsén 1991). Life event scores were also associated with recurrence and death from the disease. In a paper published in the European Journal of Clinical Hypnosis, evidence is presented that associates early childhood traumas with incidence of cancer (Harris 2006). The author points to how these traumas can have a long latency period and await a trigger in later life that can activate disease. This trauma, and the stress derived from it, is reported to cause repressed emotions that can suppress the immune system: a "time bomb" that can be defused through appropriate interventions (Harris 2006).

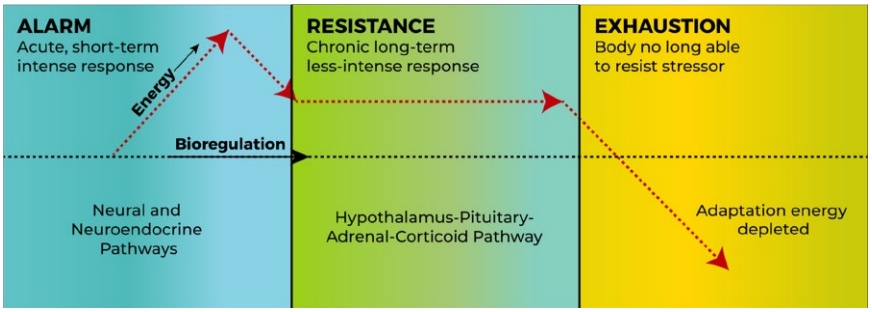


Figure 115. Selye's General Adaptation Syndrome (Selye 1946).

In the mid-20th century, Selye developed the concept of the "General Adaptation Syndrome," one of the first efforts to connect the body's ability to adapt to and heal in the face of adverse conditions (Figure 101) (Selye 1946). In this framework, he described three stages of adaptation: alarm, resistance, and, finally, exhaustion, a stage at which the body is no longer able to withstand the stressor. Because of his pioneering work, Selye was dubbed the "Father of Stress Research." This also set the stage for the work of Ader, a psychologist, and Cohen, an immunologist, who developed the field of psycho-neuro-immunology (PNI, a term used interchangeably with PNEI, which includes the role of the endocrine system in addition to the neurological and immune systems). PNI experiments established that the immune system was not as autonomous as it was made out to be but, instead, could respond to mental stimulus. In a powerful proof-of-concept demonstration in rats, Ader and Cohen showed how they could control the level of immunosuppression in the animals through prior mental conditioning (Ader and Cohen 1975, Ader et al. 1995, Ader et al. 2001).

With this underlying biological framework, the BEST Program delves into the behavioral, emotional and spiritual associations that can be contextualized in the disease experienced by the patient. Using tools such as timelines, campfire methods, and 1-on-1 counseling, BEST counselors guide patients to discover suppressed traumas that could be the root cause of disease. The ability to recognize, isolate, and eliminate these ingrained thoughts is a process, but one that we have found extremely beneficial in accomplishing seemingly miraculous recoveries.

3d. Mainstream (Conventional) Cancer Therapies

1) Chemotherapy

Chemotherapy is one of the most ubiquitous and dreaded form of cancer treatment. As a pharmacotherapy of cancer, chemotherapy utilizes substances such as alkylating agents, anti-microtubules agents, and anti-metabolites for targeting the tumor cells. Sometimes, chemo-hormonal therapies are conducted for those who are suffering from neuroendocrine cancers such as breast and prostate cancer. Mostly, these drugs are DNA recombinants to promote efficient apoptosis. By inhibiting indiscreet mitosis, they can selectively kill the cancerous cells.

However, the heterogeneity of tumor cells and the presence of chemo-resistant stem cells often requires the use of multiple toxic treatment methods to effectively treat the tumor. These high doses of chemotherapeutics also alter the function of healthy cells and can cause many adverse side effects such as myelosuppression, immunosuppression, mucositis, and alopecia (Lalla et al. 2008, Sengupta et al. 2012, Ockerby et al. 2013). They can cause diseases such as multiple sclerosis, vasculitis, systemic lupus erythematosus, rheumatoid arthritis, and vasculitis (Ben-Ari 2004). Standard short-term and long-term side effects are observed for the breast cancer patients treated with "cyclophosphamide, methotrexate, and 5- fluorouracil (CMF) or the methotrexate and 5-fluorouracil (MF) regimens" (Partridge et al. 2001). As depicted in Figure 2, some patients suffered from premature infertility or cardiac dysfunction.

Short-term effects	Long-term effects
Emesis Nausea Stomatitis Alopecia Myelosuppression Thromboembolism Myalgias Neuropathy ‡ Fatique ‡	Premature menopause/infertility Weigh gain Cardiac dysfunction Leukemia/MDS* Congnitive dysfunction †

*MDS = myelodyspastic syndrome.
† Possible long-term effect; studies are preliminary in nature.
‡ Maybe be both short-term and long-term effect.

Table 16. Side effects of chemotherapy (Partridge et al. 2001).

Toxic cancer treatments are associated with nosocomial infections by treatment-resistant pathogens such as MRSA (Avritscher et al. 2014, Rolston 2017).

These medical interventions may include chemotherapy, radiation therapy, surgery, stem cell and bone marrow transplants, immunotherapies, and targeted therapies. Commonly used devices such as catheters and ports for the administration of treatments are also likely sources of secondary infections. The majority of traditional systemic treatments, whether chemical or biological in nature, are associated with severe side effects (grades 1-4), largely because of their poor tumor specificity and high levels of toxicity (Nurgali et al. 2018, Schirrmacher 2019). Systemic and local side effects such as nausea, vomiting, diarrhea, inability to absorb nutrients, neuropathy, lymphadenopathy, memory issues, sexual changes, fatigue, and mouth sores are just some of the serious side effects from traditional therapies that can affect compliance with treatment programs as well as quality of life.

2) Radiation Therapy

Radiation therapy is typically applied synergistically alongside chemotherapy. It can be applied directly to tumors, and it impedes the growth of cancer cells by damaging the DNA of tumor tissues with radioactive particles from external beam radiation or high energy x-rays. However, it

is virtually impossible for radiation therapy to not affect healthy cells at the margins of the treatment area, possibly killing them or damaging their DNA. The accumulated radioactivity in the body also transmits systemically to cause further damage.

Some of the common side effects of radiation therapy are infertility, fibrosis, epilation, lymphedema, heart disease, cognitive decline, and radiation enteropathy (Meek 1998, Hauer-Jensen et al. 2014, Taylor et al. 2017).

3) Surgery

Surgeons use scalpels and other sophisticated technologies, such as lasers, to cut out the part of the body containing the tumor. For example, the most frequently performed treatment option for a brain tumor is a craniotomy. By cutting out the tumor area in the brain, the expectation is that the tumor will be eliminated. However, surgical treatments can have serious side effects depending on the surgical area and can cause permanent disability after surgery. In breast cancer, researchers have reported that patients complain of "arm pain on motion, anterior chest wall pain, loss of grip strength, and shoulder flexion" (Kaya et al. 2010).

4) AIC in the World of Conventional and Integrative Medicine

Given the complexity of the long-term toxicities and side effects associated with conventional therapies, it is crucial to find highly effective, yet natural and holistic treatment options. In that context, AIC has emerged as an important alternative in the non-toxic treatment of cancer that may help a patient avoid the debilitating side effects of chemotherapy.

Interestingly, AIC can be applied alongside chemotherapy and radiotherapy as well. For many years, CBHI researchers have observed cancer patients who have been treated using chemotherapy and AIC simultaneously. They found that 90 per cent of the tumor patients were free from adverse side effects of chemo and radiation with the help of AIC treatment. We surmise that AIC treatment helps the cells recover viability through regeneration of calcium depolarization by increasing

the permeability of calcium in the mitochondrial matrix.

At Hope4Cancer Treatment Centers, AIC Therapy has been an important addition to use in conjunction with other integrative cancer treatments based on the 7 Key Principles of Cancer Therapy, further avoiding the use of toxic treatment protocols.

4. Preclinical and Clinical Studies with AIC Therapy

"Evaluation of AIC Calcium for Tumor Growth Inhibition in the COLO 205 Human Colorectal Adenocarcinoma Nude Mouse Xenograft model. June 7, 2017. Charles River Discovery Services."

4a. Summary of Animal Study (Colon Cancer)

CBHI Canada contracted Charles River Discovery Services North Carolina (CR Discovery Services, https://www.criver.com) to evaluate AIC on two dosing schedules for Tumor Growth Inhibition (TGI) in the COLO 205 human colorectal adenocarcinoma nude mouse xenograft model as a feel-good trial for 28 days only.

In the Colo205-e347 study, AIC was administered orally (p.o.) at 0.83 mg/kg to two groups. One received dosing twice daily, with one dose on the first day (bid to end, beginning day one dose). The other received dosing three times daily, with two doses on the first day (tried to finish, beginning day two doses). Treatments started on Day 1 in mice with established subcutaneous COLO 205 tumors. The study included a vehicle control group, dose bid to end, first day two doses.

The study endpoint was a mean tumor volume of 2000 mm3 in the control group or 28 days, whichever came first. The study ended on Day 28. Treatment response was determined from an analysis of the percentage of tumor growth inhibition (% TGI), defined as the percentage difference between median tumor volumes (MTVs) of treated and control groups, with differences between groups deemed statistically significant at $p<0.05$ using the Mann-Whitney U Test. Tumor regressions, mean tumor growth, and treatment tolerability also were considered.

The median tumor volume on Day 28 for the control Group 1 was 936 mm3. Treatment of animals with AIC Calcium at 0.83 mg/kg, was

administered to end the first day. One dose and tried to stop, the first day two doses were associated with 0 % and 8 %, respectively. Treatments administered in the study were acceptably tolerated based on the group mean BW measurement and clinical observations.

4b. Chronic Leukemia and Multiple Myeloma Clinical Case Studies

After treating with AIC for a certain period of time, patients have shown significant improvement, especially those who are suffering from bone-related cancer. We report here the results from two patients: Ms. Holt, a 78-year-old Caucasian female diagnosed with multiple myeloma, and Mr. Kim, a 72-year-old male patient diagnosed with chronic leukemia. Both patients authorized the release of their medical data reported here.

Both patients showed notable improvement after treatment with AIC with a complete recovery from cancer after only being treated with AIC. As it is shown in the data, the white blood cell (WBC), hemoglobin, platelet and neutrophil concentrations have increased considerably in 4 years. For Mr. Kim's case, the WBC, RBC, neutrophils, lymphocytes and hemoglobin concentrations significantly increased within the first month of treatment.

5. Conclusion

By analyzing the current trend of treatments for aging-related chronic degenerative diseases including osteoporosis and cancer, it became clear that all these diseases are related to the dysfunction of calcium influx and efflux. As the flow of ionized calcium is altered, it is calcified in the mitochondrial matrix, which results in intensified production of Reactive Oxygen Species (ROS). Moreover, as the calcium homeostasis system is deconstructed, Bone Mineral Density (BMD) and the bone turnover rate are altered, which ultimately causes the deterioration of bone health.

Through the research, we have found that AIC can be a practical treatment option for treating chronic degenerative diseases as it balances calcium homeostasis, and initiates calcium signaling at both of the extracellular and intracellular levels. As Choi and co-workers have investigated,

AIC has a notable efficacy in elevating BMD as established with their animal experiment (Choi et al. 2011). The extremely high rate of increase of BMD with AIC treatment for ovariectomized rats for 12 weeks indicated the possibility of using AIC to reverse osteoporosis. Moreover, AIC is also used to treat chronic neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), Parkinson's Disease and Alzheimer's Disease. As calcium modifies mitochondrial function by balancing up the intracellular calcium level, it can decrease ROS production.

Moreover, it has been shown that AIC can help to initiate the activity of Mesenchymal Stem Cells (MSC) by regenerating mitochondrial function. As more ATP is produced and MSCs are activated by the depolarization caused by the influx of calcium ions, it can stimulate their cell division. As MSC is initiated, it can be the key to treating chronic diseases. For example, as MSC in bone marrow is activated, it can transform to chondrocytes, which can help to manage osteogenic arthritis.

Furthermore, as calcium enhances the activity of mesenchymal stem cells, it helps to develop dental bone health, which is helpful to rebuild dental bone after dental implants. It is known that calcium deficiency and unbalanced levels of ionic calcium can contribute to the risk of developing dental gum and periodontal diseases. Therefore, as the efficacy of AIC has been proven to balance extracellular and intracellular calcium levels, the intake of AIC calcium is recommended to prevent the growth of bacteria in the gums and improve gingivitis and periodontal health.

Also, as AIC modifies mitochondrial function, it can adjust protein synthesis, which helps to produce p53 protein. Also, by secreting apoptosomes such as cytochrome c, it can help to effectively target the cancer cells when it is introduced into the cell. Moreover, as AIC neutralizes pH level by forming calcium lactate and calcium ammoniate, it can prevent cancer cells from being proliferated in the cell. Furthermore, as calcium reduces TXNIP protein expression by inhibiting the production of glutamine, AIC can be a treatment for cancer.

The case studies have shown that AIC has notable efficacy in treating cancer. Ms. Holt and Mr. Kim's data demonstrate the likely effects of AIC. As they are completely recovered from cancer by taking AIC, it is hard to deny the efficacy of AIC while the clinical data provides proof of its effects.

Moreover, as their hematological data has been significantly improved, it would be a wise idea to try AIC as a cancer treatment option. While currently used cancer treatments have many adverse side effects, it is essential to find an alternative option that can enhance overall body metabolism.

Through the thoughtful, systematic literature review, it became more evident that AIC can be an innovative way of approach to treating chronic degenerative diseases. Even though there has been limited research conducted to date around the efficacy of AIC, it is crucial to investigate more about the effects of AIC while it shows significant improvements when applied to patients with chronic degenerative diseases, such as osteoporosis and cancer.

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XII. SAC and Dental Bone Health

"How is it Possible to Increase Jawbone BMD Without Bone Grafting? SAC Calcium Therapy Can Be an Option for Enhancing Dental Bone Health by Maintaining Periodontium and Pulpitis Healthily."

"It can be said that SAC Calcium therapy is an innovative treatment that can increase bone density and improve periodontal tissue and dental pulp health without bone grafting."

"I received a call from an acquaintance not long ago. He said that he had a molar tooth removed, and the attending doctor told him that the only way to implant a tooth is bone grafting. He himself did not want bone grafting, but the doctor said that he must have a bone graft. Otherwise, the bone tissue could not regenerate.

Ultimately, I was very frustrated, but I suggested taking SAC Calcium for five months. Five months later, he went to the dental clinic for an X-ray examination, and the results were surprising. The originally depressed bone tissue had regenerated to the extent that it could accept tooth implantation."

"A few years ago, a man in his 40s visited the laboratory. At that time, his molars were loose, there were no salvageable molars at all, and the state of his gums was very poor. The dentist said that he could not even try dental implants.

Therefore, he merely recommended pulling out all of the remaining teeth and installing dentures. In addition, a considerable part of the upper and lower jaw bone tissue had been lost due to dental inflammation. I felt that the only way to help him was with SAC Calcium, so I recommended taking SAC Calcium for six months. Surprisingly, when he visited the research center a month later, his dental condition had improved significantly. He said that within a month, he had recovered from the inflammation in the gums and dental diseases, and that he no longer experienced any inconveniences in using his teeth. "

1. Introduction

With the advancement of medicines and dental technologies, the life expectancy of the world's population has considerably increased over the past few decades. As rising numbers of people live longer, the quality of life of the elderly has gained much attention along with demands for improvement. With growing concerns about age-related diseases, oral health also accounts for a large part of the quality of life of the elderly.



Figure 116. Dental Bone Health Relates to Quality of Life.

Edentulism in the elderly population around the world is between 11% and 44%, inversely increasing with socioeconomic status. (Gaetti et al., 2011) The use of implants is considered to be one of the most popular alternatives for its high predictability, durability, and functionality in rehabilitating patients aesthetically and functionally. (Gaetti et al., 2011) Moreover, loss of alveolar bone due to periodontitis is a significant concern for all age groups, as it supports not only possible implants, but also natural teeth. (An et al., 2012; Bosshardt, 2005)

2. Type 2 Diabetes and Dental Bone Health

It is also very critical to factor in that a growing number of patients with systemic disorders, such as diabetes mellitus, seek dental treatment. Such patients generally experience an increased risk of osteoporotic fractures, and depending on the type of diabetes, bone formation and osteoblast function are impaired. (Hamann et al., 2012) Type 2 diabetes patients may have increased BMD. However, their bone quality is significantly reduced. (Hamann et al., 2012) A study done by Abbassy, Watari & Soma

(2010) suggests that uncontrolled diabetes mellitus decreases mandibular bone formation, reduces the rate of bone turnover in the alveolar wall, and affects the quality of bone. (Abbassy, Watari & Soma, 2010)

3. Current Issues When Considering Dental Implants

When considering dental implants, there are two major types of treatment. For many years, subperiosteal implants were used successfully. However, this solution is no longer favored for its significant disadvantages, such as its substantial surgical procedures, extensive fabrication requirement, and superstructure castings. (Von Fraunhofer, 1976) Even with endosseous dental implants being a significant part of prosthodontics and restorative dentistry and its advances in technology, the concern for potential clinical failure is still substantial for both dentists and patients. (Porter & Von Fraunhofer, 2005) In response to the interests of treatment implications, although, prosthetic design mainly governs the surgical placement of the endosseous implant, morphology, and quality of the alveolar bone are crucial to its success. (Wikesjo et al., 2001) Also, the alveolar processes of the maxilla and mandible provide support for the teeth, and the changes in these bones by osteoporosis affect tooth retention and the stability of dental implants as well as alveolar bone height. (Gaetti, 2011; Wactawski, 1996)



Figure 117. Dental Implants and Bone Health.

4. The Relationship between Calcium and Periodontal Ligament Cell Facilitation

Another crucial factor to consider in dental health is human periodontal

ligament cells (hPDLCs). Human periodontal ligament cells reside within the periodontal ligaments and can differentiate into either cementoblasts to synthesize root cementum or osteoblasts to synthesize alveolar bone. (McCulloch, 1996) Koori et al. (2014) investigated the effects of extracellular calcium on the cell proliferation and osteogenic differentiation of human periodontal ligament cells (hPDLCs). In the human PDLSC line they examined, calcium treatment increased the expression of genes of bone-related molecules, such as BMP-2, OCN, OPN, and RUNX2, and also resulted in mineralization. (Koori et al., 2014) These genes were found to facilitate periodontal ligament cells (Ivanovski et al., 2001), and be regulated by RUNX2. (Lee et al., 2000) Also, increased concentrations of Ca^{2+} and P were observed to have an enhancing effect on the cell proliferation, differentiation, and mineralization of hPDLCs. (An et al., 2012)

5. SAC as a Treatment for Enhancing Dental Bone Health and Gum Health

In an effort to apply the effects of SAC to the restoration of the periodontium, we emphasize its outcome on the systemic regulation of hormones associated with calcium homeostasis. It is widely known that significant regulators of calcium homeostasis, and therefore of bone metabolism, are parathyroid hormone (PTH) and thyroid hormone (TH). (Liu et al., 2016) As mentioned earlier, recombinant parathyroid hormone (PTH) is currently used as an alternative to anti-resorptive treatments for osteoporosis. In a rabbit model of osteoporosis, Bellido et al. (2010) show that administration of PTH almost wholly reversed the decline in bone mass density and calcium content in the peri-alveolar region, thereby reducing global mandibular bone loss. (Bellido et al., 2010)

6. SAC as a Trigger for Calcium Homeostasis: The Key to Improving Dental Bone Health and Gum Health

Moreover, the experimental study done by Li et al. (2017) suggests that PTH also promotes cementum formation by activating several cementogenesis- and differentiation-related biomarkers. Cementum formation and its repair process by cementoblasts (Bosshardt, 2005) are essential,

as they play a crucial role in the development of functional periodontal ligaments. (Li et al., 2017) It was found that intermittent low-dose PTH enhanced the mineralization capacity of cementoblasts and therefore promoted cementum regeneration. (Li et al., 2017) However, it must be noted that dosage and duration of administration are essential details, as PTH can apply both anabolic and catabolic effects on bone. (Liu et al., 2016; Kasagi & Chen, 2013; Li et al., 2017) SAC initiates systemic regulation of both the thyroid hormones and parathyroid hormones involved in calcium homeostasis which, in turn, stabilizes the absorption and resorption of bones. Therefore, the systemic control of thyroid hormones by SAC can efficiently be used to restore the loss of bone in the peri-alveolar region as well as cementum formation in the periodontal area.

7. SAC as an Initiator to Stimulate Mesenchymal Stem Cells for Growth of Dental Bone and Gums

There exist many pieces of research that reiterate the importance of calcium ions and their balance in maintaining and reviving bone health. In many cases, synthetic bone replacement is used to fill and restore alveolar bone. (Caffesse, 2002) Targeting the underlying condition of calcium and hormonal imbalances is essential. SAC as a physiological calcium ion triggers the systemic regulation of hormones and therefore calcium homeostasis and revives core metabolism. Moreover, as Egusa et al. (2012) have found, "crosstalk between implanted donor cells and recipient immune cells plays a key role in determining clinical success that may involve the recently observed immunomodulatory properties of mesenchymal stem cells (MSCs)." (p. 229) Also, they added, "Ca/P-based biomaterials have significant potential for bone regeneration." (p. 241) Therefore, recommending additional calcium supplement intake for patients who are considering dental implants is crucial; as it improves dental bone regeneration.

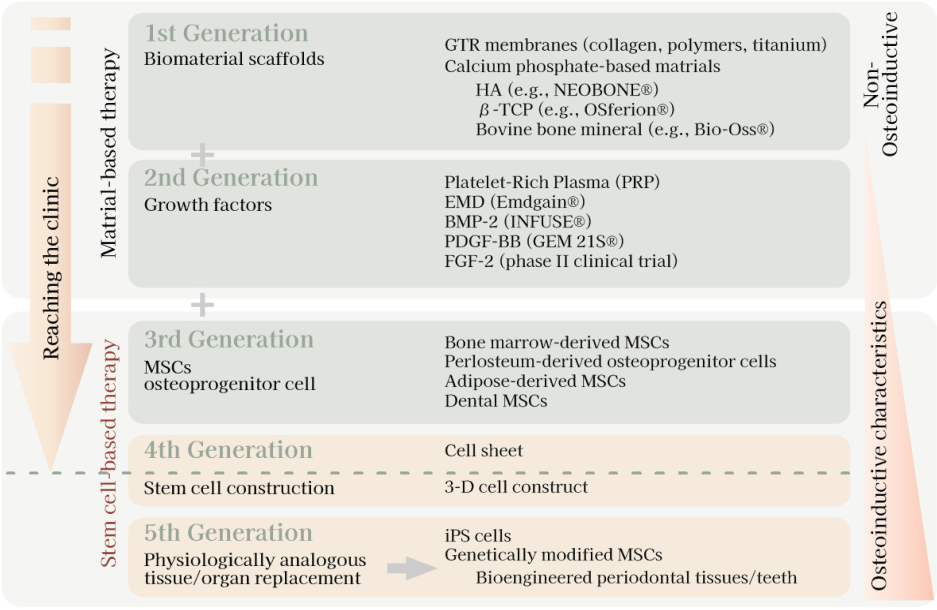


Figure 118. Progress in Regenerative Periodontal/Bone Therapies. Regenerative periodontal/bone therapies are broadly categorized as material-based therapies (first-generation biomaterial scaffold-based approach and second-generation growth-factor-based approach) and stem-cell-based therapies (third-generation MSC/osteoprogenitor cell-based approach, fourth-generation stem-cell construction-based approach, and fifth-generation physiologically analogous tissue/organ-replacement approach). Adapted from "Stem cells in dentistry – Part II: Clinical applications," by H. Egusa et al., 2012, Journal of Prosthodontic Research, 56 (4), p. 231. Copyright 2012 by Elsevier Ireland.

8. Case Study for SAC Treatment in Dental Bone Health

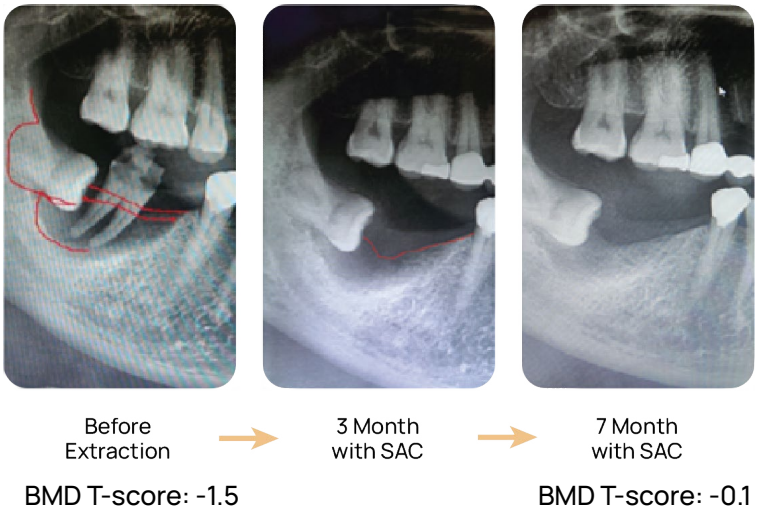


Figure 119. X-ray Image for an Asian Male Who is 58 Years Old, Conducted Molar Tooth Extraction; Dental Bone Growth After Treatment with SAC for 7 Months without any Cotreatment.

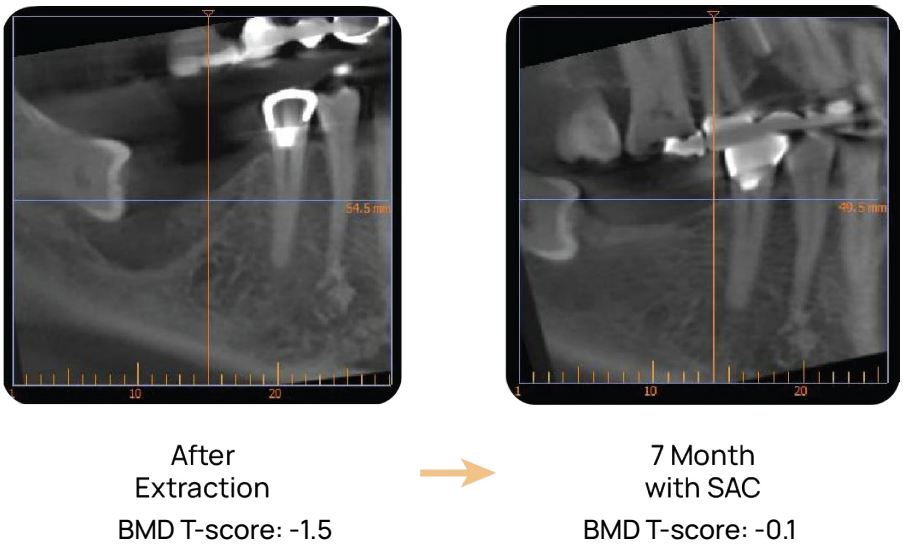


Figure 120. X-ray Image for an Asian Male Who is 58 Years Old, Showed Dental Bone Growth After Treatment with SAC for 7 Months without any Cotreatment. (His Dental Bone T-score Showed Significant Improvement)

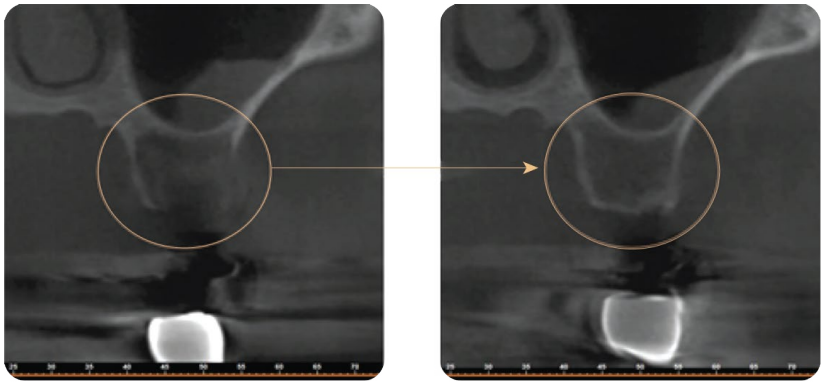


Figure 121. X-ray Image for an Asian Male Who is 58 Years Old, Showed Dental Bone Growth After Treatment with SAC for 10 Months without any Cotreatment. (His Dental Bone T-score Showed Significant Improvement)

9. SAC and Gingivitis / Periodontal Disease (Gum Disease)

"Inadequate Calcium Levels Result in Severe Gum Disease. So, Through Treatment with SAC, Dental Bone Health Can be Improved."



Figure 122. Gum Disease Image.

It is well-known that one-third of the population in developed countries are suffering from gum disease and periodontal disease. (ADHA, 2008) Both oral health and general health are affected by the food you choose to eat and how often you consume it. Bacteria cause gingivitis or periodon-

tal dental disease, which can destroy the tissue that surrounds the teeth. The difference between gingivitis and periodontitis is that periodontitis is usually preceded by gingivitis; but not all gingivitis progresses to periodontitis. In the early phase of gingivitis, the bacteria in plaque causes inflammation in the gums to bleed easily, even while simply brushing the teeth. (Abbas et al., 1986) The teeth are still firmly planted in their socket even though the gums may be irritated. In this phase, there are no evident signs of irreversible bone damage or other tissue damage. Gingivitis advances to periodontitis when it is not treated. Prominently seen in periodontitis patients is that the inner layer of the gums have pulled away from the teeth and formed pockets. The pockets formed between the teeth and gums collect debris and can cause infection. The immune system fights the bacteria, which grows beneath the gum line.

10. The Causes of Gum Disease and SAC Treatment

Plaque is the primary cause of gum disease. However, changes in hormones during pregnancy, hygiene, menopause, and menstrual periods can cause gums to be sensitive, which can lead to periodontal disease. (Rajesh et al., 2016) Moreover, diseases like HIV and cancer can interfere with the function of the immune system.

Type 2 diabetes patients are especially vulnerable to gum disease because the disease is affected by fluctuations in sugar uptake. (Mansour & Abd-Al-Sada, 2005) Therefore, type 2 diabetes patients are at higher risk of developing infections including periodontal disease and cavities. Medications can interfere with oral health, because some reduce the flow of saliva, which has a protective effect on teeth and gums.

Drugs like anticonvulsant medications, “Dilantin and Adalat can cause abnormal growth of gum tissue.” (Darley, n.d.) Moreover, smoking can prevent gum tissue from repairing itself. Without consistent effort to brush and floss, gingivitis can quickly develop. (Drocolinrichman, 2014) A family history of dental disease can have a bearing on the development of gingivitis. (Medical News Today, 2018) To date, many gingivitis and periodontal disease treatments have made their way into the market, but these have not yielded any remarkable improvement in the condition of the patient.

Calcium deficiencies or an imbalance in ionic calcium levels can be contributing factors to gum and periodontal diseases. Therefore, SAC Calcium helps balance calcium levels to prevent the growth of bacteria in the gums and improve the gingivitis and periodontal health.

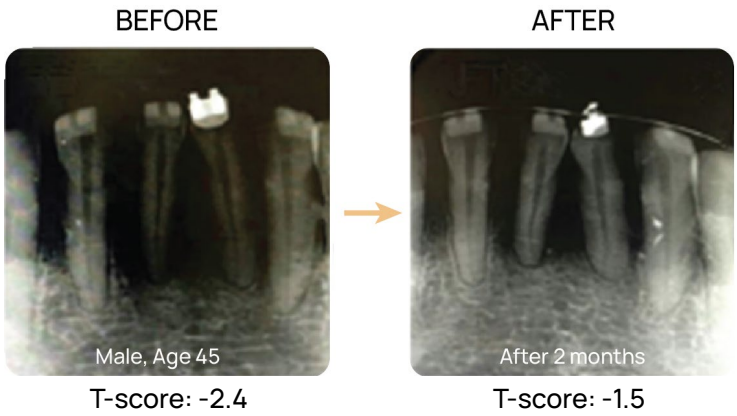


Figure 123. X-ray Image for an Asian Male Who is 45 Years Old, Showed Dental Bone Growth After Treatment with SAC for 2 Months with Splint Only. (His Dental Bone T-score and Poor Gum Health Showed Significant Improvement)

SAC Calcium Releases Bioactive Physiological Ionic Calcium to

- Strengthen Jaw Bones.
- Rebuild Periodontium.
- Stimulate Bone Formation.
- Boost Bone Remodeling.
- Enhance Gum Health.
- Boost Mineralization.

XIII. SAC Calcium and Diabetes

"There is a hormone in the bone metabolism index called osteocalcin. Osteocalcin plays a role in secreting adiponectin, a peptide that inhibits insulin resistance. SAC Calcium improves bone quality and bone density while helping to increase bone calcium."

"A long-term type 2 diabetes patient sent us an e-mail after taking SAC Calcium for nearly seven months. His blood sugar level on an empty stomach was 29-30 mmol/L, but after he started taking SAC Calcium, it dropped to 17 mmol/L. After five months, it was under 7 mmol/L. He experienced the miracle of maintaining normal blood sugar levels after seven months."

1. Introduction

Diabetes, known as the modern disease, is no longer a strange disease. Due to the intake of refined carbohydrates, overnutrition, Westernized eating habits and insufficient exercise, the number of diabetic patients is on trend to increase significantly (IDF, 2017). According to the International Diabetes Federation, based on figures from 2017, about 425 million people in the world have diabetes, and it is predicted that about 630 million people will have diabetes in 2045 (IDF, 2017). In addition, 4 million diabetic patients die every year due to complications of diseases such as coronary heart disease, cerebrovascular disease, and foot & bowel diseases (IDF, 2017). It is said that the annual medical expenses associated with diabetes has reached 72.7 billion US dollars, which is equivalent to 12% of the total medical expenses among people aged 20 to 79 (IDF, 2017). If there is a genetic history of type 2 diabetes, the probability of developing diabetes is three times higher (Flores et al., 2003; Hansen, 2003; Gloyn, 2003). But so far, the cause of hereditary diabetes has not been accurately identified.

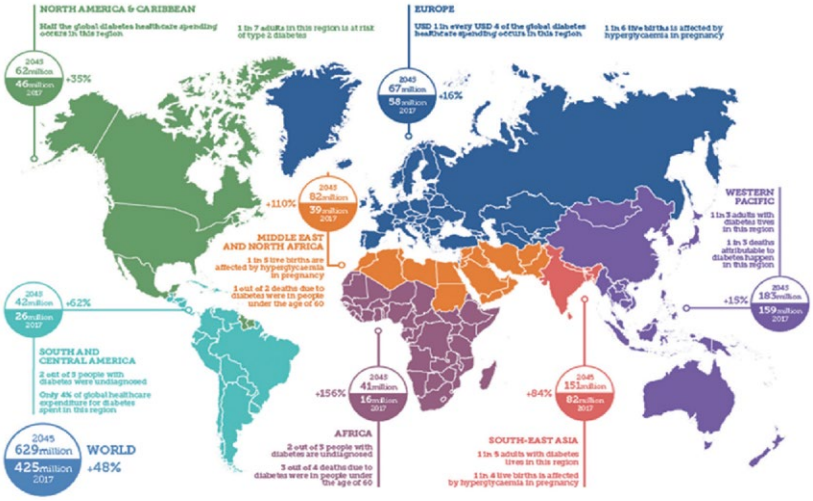


Figure 124. Global Diabetes Statistics

The World Health Organization diagnoses diabetes based on fasting blood glucose, normal plasma blood glucose, glycosylated hemoglobin, and blood glucose values two hours after oral glucose (Goldenberg & Punthakee, 2013).

What are the diagnostic criteria for hyperglycemia?

Fasting plasma glucose concentration	≥ 7.0 mmol/l (126 mg/dL)
Same as the glucose tolerance test, the plasma glucose concentration after oral administration of 75 g glucose for 2 hours	≥ 11.1 mmol/l (200 mg/dL)
Symptoms of hyperglycemia and usual plasma glucose concentration	**≥ 11.1 mmol/l (200 mg/dL)**
Glycated hemoglobin (HbA1c)	≥ 48 mmol/mol ≥ 6.5 DCCT (Diabetes Control and Complications Trial)%

Table 17. Diabetes Diagnostic Criteria Defined by the World Health Organization

If you have any of the above conditions, you will be diagnosed as having hyperglycemia symptoms (Alberti & Zimmet, 1998), and if you exceed

126 mg/dL in two fasting blood glucose tests, you will be diagnosed as having diabetes (WHO, 2006)

Diabetes, as a hyper glycated hemoglobin, is a metabolic disease with persistently high blood sugar states in the blood. First, this is a pancreas problem, which is caused by the failure to successfully produce and release insulin (Abiola, Sathyapalan & Hepbum, 2016). Once food is ingested, blood sugar will rise, and the body will respond to it, producing insulin from pancreatic beta cells and releasing it into the blood (Sonksen & Sonksen, 2000). Under the action of insulin, glucose turns into glycogen and plays a role in lowering blood glucose values (Lopez, Gomez-Lech & Castell, 1991). But if the pancreas cannot perform its functions normally, it cannot secrete insulin smoothly.

Second, citation occurs due to the failure of the insulin receptor and insulin to bind normally, which is one of the causes of insulin resistance. When insulin binds to the insulin receptor, the glucose transporter (glucose transporter-4) in the cell moves to the vicinity of the cell membrane to promote the flow of glucose from the outer layer of the cell into the interior. With type 2 diabetes, insulin can be adequately supplied, but cannot bind to the receptor, so blood glucose levels are maintained at a high level. Choo & Ko (2006) believe that the mitochondria disease in muscle results in the reduced oxidation capacity of fatty acids, and the inability to oxidize acetyl CoA and diacylglycerol (DAG), activate protein kinase C, and bind to insulin. Therefore, insulin cannot connect with the receptor and eventually hinders the absorption of glucose (Choo & Ko, 2006).

The above factors lead to the inability to lower elevated blood sugar. Accompanied by kidney disease, hand and foot paralysis, severe cases will develop into Alzheimer's disease (Moller, 2001; Marx, 2002; Janson, 2004).

The initial symptoms of diabetes include polydipsia, polyuria, polyphagia, weight loss, etc. If, after experiencing these symptoms, treatment for diabetes is not received, they will induce other complications. Acute complications include hyperosmolar hyperglycemic nonketotic syndrome (HHNS) and diabetic ketoacidosis (Umpierrez, 2006); chronic complications include cardiovascular disease, chronic renal failure, stroke, diabetic ulcers, osteoporosis, diabetic retinopathy, etc. (Kanda et al., 2008; Mehdi & Toto, 2009; Lim et al., 2016).



Figure 125. Complications Caused by Diabetes

2. Classification and Causes of Diabetes

Diabetes is divided into three types, type 1 diabetes, type 2 diabetes and gestational diabetes. Type 1 diabetes can occur at any age, but mainly occurs during childhood (Pociot & Mcdermott, 2002). The cause of type 1 diabetes has not yet been correctly identified. With type 1 diabetes, due to genetic, immune and environmental factors, the beta cells of the pancreas are destroyed and insulin cannot be fully secreted.

According to Belle, Copperieters & Herrath (2011), type 1 diabetes is caused by the body's own immune system. The β cells of islets of Langerhans are destroyed or damaged, resulting in decreased insulin production. According to the research of Pociot & Mcdermott (2002), there are about 20 different chromosomes associated with type 1 diabetes.

So far, the most common treatment for type 1 diabetes is the method of regulating blood sugar levels by injecting insulin. Type 2 diabetes is caused by Western-style eating habits and insufficient exercise. It is a metabolic disease caused by insulin resistance and abnormal insulin secretion. Type 2 diabetes accounts for 90% of diabetic patients (WHO, 2013).

Irregular eating habits, a high intake of refined rice, saturated fat, and trans fat can increase the incidence of type 2 diabetes (Riserus, Willett & Hu, 2010; Hu et al., 2012). Choo & Ko (2006) stated that lipids such as acetyl-CoA, or diglycerides that do not form material metabolism, induce the phosphorylation of insulin by activating protein kinase C, which prevents cells from using insulin.

Finally, gestational diabetes, which is similar to type 2 diabetes, is a disease caused by inconsistent insulin secretion and response. According to surveys, 5-10% of pregnant women develop diabetes, most of which are type 2 diabetes (NDSR, 2017). It is a temporary disease, but not receiving treatment will damage the health of the mother and fetus.



Figure 126. Drugs Prescribed for Diabetic Patients

3. Diabetes and Osteocalcin

"A study by Dr. Cassenti of Columbia University in the United States showed that the osteocalcin protein formed in osteoblasts induces insulin secretion and regulates blood sugar. A published paper documented the fact that SAC Calcium is effective for osteocalcin secretion, so we need to pay full attention to SAC Calcium treatment of diabetes."

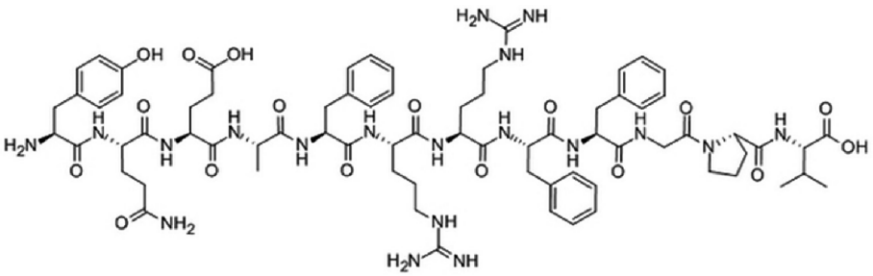


Figure 127. The Structure of Osteocalcin Produced in the Body

A disease closely related to diabetes is osteoporosis (Wongdee, 2011). Jackuliak & Payer (2014) stated that diabetic patients are six times more

likely to experience fractures due to bone loss than the average person. Diabetic patients have a high incidence of fractures due to osteoporosis. According to the type of diabetes, it can be observed whether bone formation and the function of osteoblasts have been damaged (Paschou et al., 2017). Although the bone density of type 2 diabetic patients may have increased, the probability of fractures is 2 to 3 times higher than that of normal people due to the significant reduction in bone quality. (Saito, 2013) Ferron et al., (2012) pointed out that the metabolism of glucose is closely related to bone formation. Osteocalcin, which is produced by osteoblasts in bones, is responsible for the dual function of regulating insulin production and insulin sensitivity. This has been confirmed by mouse experiments and test tube experiments (Ferron et al., 2012).

According to an experiment by Kanazawa (2015), mice were injected with osteocalcin (recombinant osteocalcin) and results confirmed that glucose resistance was improved and insulin expression in β cells was increased. The research results of Kindblom et al. (2010) also show that the value of osteocalcin is inversely proportional to the value of plasma glucose. Real et al. (2009) proved that the value of plasma osteocalcin is associated with insulin resistance.

The role of osteoblasts and glucose metabolism are further explained below.

Osteoblasts produce osteocalcin and then undergo carboxylation (carboxylate) (Patti, 2013). As a result, the carboxylated osteocalcin (GLA-OC, or cOc) of the bone matrix is accumulated (Lacombe, 2015), which then causes bone exchange. At that time, the osteocalcin hydroxylated by osteoclasts was converted back into undercarboxylated osteocalcin (GLU-OC, or UcOc) (Ferron et al., 2012). UcOc helps the expression of glucagon-like peptide-1 (GLP-1) from the enteroendocrine cells of the small intestine (Ferron et al., 2012). These processes prove that glucose metabolism and bone exchange are closely related, and osteocalcin has become an important “key” for the treatment of type 2 diabetes.

4. Bone Density and Diabetes

Through long-term testing of bone mineral density in diabetic patients, it was unexpectedly found that some people have very low bone density or high T-scores and Z-scores (Cakmak et al., 2015). According to the type of diabetes, type 1 diabetes patients have low bone mineral density (Vestergaard, 2007), while type 2 diabetes patients have high bone mineral density (Dutta, Pakhetra & Garg, 2012). Although there are some differences between type 1 diabetes and type 2 diabetes, they both are associated with a higher incidence of fractures (Sundararaghavan et al., 2017). As mentioned before, decreased bone metabolism in diabetic patients leads to poor bone health, and the low secretion rate of osteocalcin leads to increased bone deterioration (Saito, 2013). Therefore the common denominator in the two types of diabetes is that blood glucose levels are both very high.

In essence, high blood glucose levels produce advanced glycation end products (AGEs) (Sundararaghavan et al., 2017). The substance is formed by the combination of the carbonyl group of sugars and the amino group of proteins, which induces the apoptosis of mesenchymal stem cells, hinders the differentiation of osteoblasts, and causes fatal trauma to bone health (Yamagishi et al., 2005). The advanced glycation end products ultimately increase the activity of osteoclasts and cause bone loss (Zhou et al., 2006). In addition, the high blood glucose levels of diabetic patients hinder the formation of osteocalcin by osteoblasts, thereby inhibiting bone formation (Inaba et al., 1995).

Type 1 diabetes causes a decreased bone density value, which has an adverse effect on the formation of bone microstructure (bone microarchitecture). It induces bone marrow mesenchymal stem cells to differentiate into adipocytes and hinder the differentiation of osteoblasts (Moseley, Doyle & Beur, 2018). In addition, the decrease of insulin-like growth factor 1 (IGF-1) and insulin receptor also leads to a reported decrease in bone formation (Lu et al., 2003).

Type 2 diabetes and type 1 diabetes look different. Compared with type 1 diabetes, which results in reduced bone density, type 2 diabetes causes an increase in bone density (Sundararaghavan et al., 2017)). Ma et al., (2012) observed the results of 3437 diabetic patients, and showed

that the bone mineral density of type 2 diabetic patients exhibited a very surprising increase. The reason is that insulin levels in the blood of type 2 diabetic patients is very high, which contributes to the action of osteoblasts and induces bone formation. Some people will ask whether increased bone density is better? However, if there is no bone exchange, but only bone formation, bone quality will deteriorate, increasing the probability of fractures (Melton et al., 1997). According to a study by Rubin & Patsch (2016), the results of examinations for elderly people with type 2 diabetes show that the probability of fractures is 50 to 80% higher. In order to form healthy bones, bone exchange and bone formation must be developed in a balanced manner. However, patients with type 2 diabetes cannot form bone exchange due to insufficient osteocalcin (Bothy, Hermans & Gruson, 2016)

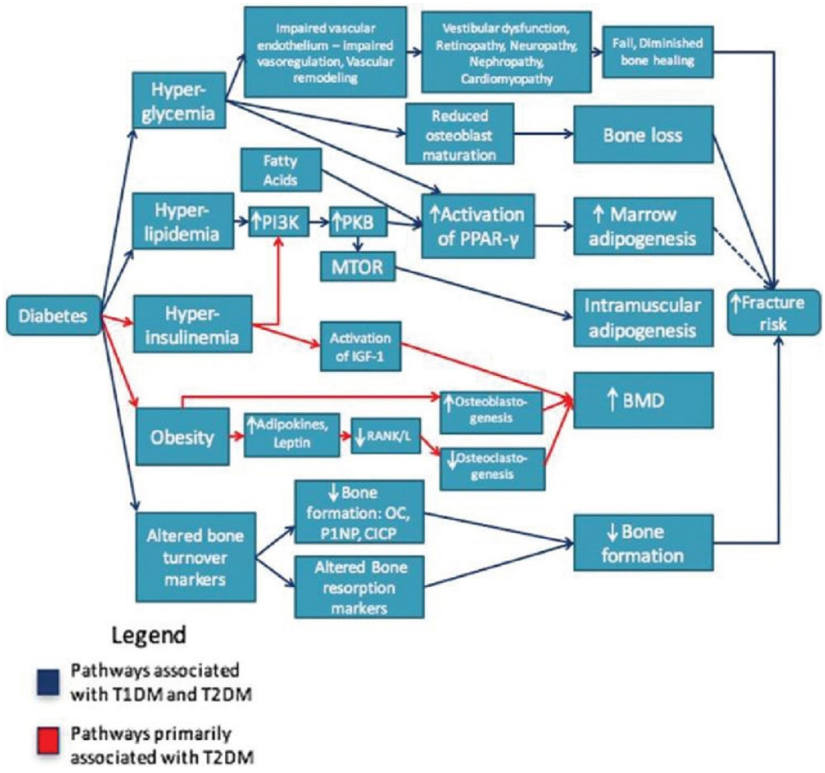


Figure 128. The mechanism by which diabetes increases fracture risk, excerpted from "Diabetes and Bone Health: Latest Research and Clinical Trials", by Sundararaghavan et al., 2017, Therapeutic Advance in Musculoskeletal Disease, p. 69. Copyright 1965 by SAGE Journals

5. Types and Effects of Modern Diabetes Treatment Agents

5a. Metformin

Metformin is mainly an oral-administration type diabetes treatment agent prescribed for patients with type 2 diabetes. The guide book issued by the American Diabetes Association (2013) advises the use of a dimethyl double arc first. In type 2 diabetes patients, compared to normal people, there are three times more glucose nascent processes (Hundal et al., 2000). Metformin shortens the process of glucose regeneration to one-third, and ultimately prevents the liver from producing glucose, so as to reduce blood sugar (Hundal et al., 2000).

To expound in greater detail, the dimethyl double arc guides the production of the AMPK enzyme (Zhou et al., 2001). AMPK inhibits the development of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, which are involved in the process of glucose regeneration and inhibits the production of glucose. It also prevents glucagon from breaking down into glucose. Not only that, it also plays a role in improving insulin sensitivity, so it is widely used in the treatment of diabetes.

According to research by Razavi et al., (2017), metformin can cause side effects such as diarrhea, cramps, nausea, vomiting and gastrointestinal irritation, and occasionally severe lactic acidosis is also found in patients (Haloob & Zoysa, 2016). The liver of normal people uses lactic acid to act on the gluconeogenesis reaction of glucose, while in patients injected with dimethyl double arc, no glucose synthesis reaction of glucose occurs in the liver, and lactic acid continues to remain in the blood. According to a report by Reinstatler et al., (2011), patients taking metformin have a reduced ability to absorb vitamin B12, which leads to anemia caused by vitamin B12 deficiency.



Figure 129. Metformin

5b. Incretin

Incretin is not intended to alleviate temporary symptoms, but is a medicine used to restore patients to normal life through medium- and long-term treatment. Incretin is also known as glucagon-like peptide-1 (GLP-1) or glucose-dependent insulinotropic polypeptide (GIP). The most common incretin substances are liraglutide and pramlintide. According to the latest animal experiments (Brubaker & Drucker, 2004), glucagon-like peptides increase the size of islets of Langerhans of the pancreas and increase the proliferation of β-cells. Experiments in mice have shown that the glucose sensitivity of mouse β-cells injected with glucagon-like peptide is significantly increased (Kiihtreiber, Habener & Holz, 1993).

It has been observed in patients with type 2 diabetes that they usually have higher glucagon values (Quesada et al., 2008). Therefore, glycogen stored in the muscles is broken down into glucose, resulting in a high glycemic index in the blood (Lopez, Gomez-Lech & Castell, 1991). When incretin is injected, it reduces the glucagon index in the body and preemptively prevents a rise in blood sugar (Kim & Egan, 2008). At the same time, it increases the amount of insulin secreted by the body and balances the insulin/glucagon ratio in the arteries (Chia & Egan, 2008). This can suppress the amount of glucose produced in the liver.

However, the mechanism has not yet been fully understood, and it is not clear what effect it has on organs that have not become the target. Incretin is rapidly decomposed in the body by dipeptidyl peptidase degrading enzymes, and it only takes two minutes to act in the body (Chia & Egan, 2008). Incretin is rapidly inactivated by dipeptidyl peptidase (DPP-4), and now scientists are studying ways to inhibit dipeptidyl peptidase

(DPP-4). However, it is predicted that dipeptidyl peptidase (DPP-4) will cause the side effects of related antigen enzymes, such as immune regulation and cell death, on the cell surface.

Amori, Lau & Pittas (2007) compared the results of the experimental group injected with incretin and the control group, and found that 57% of the experimental group injected with incretin had nausea and 17% had vomiting symptoms. In addition, because it is a blood sugar lowering ingredient, it can cause hypoglycemia symptoms (Seino, Kuwata & Yabe, 2016).

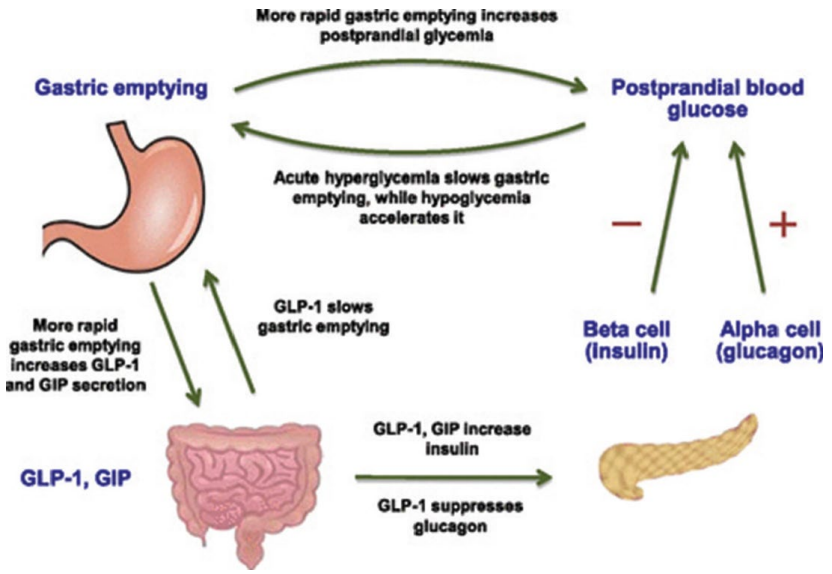


Figure 130. The interdependence of gastric output, incretin hormone and postprandial blood glucose, excerpted from "The relationship between gastric excretion, postprandial blood glucose and incretin hormone", by Marathe et al., 2013, Diabetes care, p. 69. Copyright 2018 by the American Diabetes Association

5c. Sulfonylurea

Sulfonylureas are mainly prescribed to patients with type 2 diabetes. The most common drugs include glimepiride, glibenclamide, gliclazide, glipizide and so on. Sulfonylureas are drugs that promote insulin secretion in pancreatic β-cells (Proks et al., 2002). To expound in detail on the mechanism of action, sulfonylurea binds to the ATP-dependent potassium pump on the pancreatic-β cell membrane (Proks et al., 2002), so that

the internal potassium does not flow out.

Outside the cell, the inner membrane potential rises (Proks et al., 2002). As a result, an action potential is generated, and calcium channels are opened to allow calcium to flow into the cell. Through this process, β cells are activated, and insulin secretion increases, thereby regulating blood sugar.

However, various side effects have been found in patients using sulfonylurea. According to a survey by Yanping et al., (2014), excessive insulin secretion causes hypoglycemia, in addition to indigestion, headaches, and allergies (Yanping et al., 2014).

6. SAC Calcium, Osteocalcin and Diabetes

Many of the aforementioned documents have shown the relationship between osteocalcin and diabetes. Nevertheless, there is currently no established method for the treatment of diabetes by means of calcium and osteocalcin.

The bone metabolism of osteoblasts and the glucose metabolism of insulin are closely related to osteocalcin (Ferron et al., 2010). Thus, as long as the secretion of osteocalcin can be increased, the patient's blood glucose can be adjusted. SAC Calcium directly enters the body's plasma and participates in the body's hormone action and calcium homeostasis. Therefore, by ingesting SAC Calcium to increase the amount of plasma calcium, the effect of osteocalcin can be induced, and the blood sugar level of diabetic patients can be adjusted. There is no need to worry about hypoglycemia caused by other diabetes treatment agents.

The experiment of Choi et al. (2011) found that SAC Calcium fed to mice had the effect of reducing the loss of osteocalcin in the bones. These test results prove that SAC Calcium can activate osteoblasts, and it can be inferred that the amount of osteocalcin increases by at least 14-19% in the bones. It is believed that SAC Calcium could be a major turning point in the treatment of diabetes.

SAC Calcium therapy is not only effective for osteocalcin, but also has an important impact on the action of pancreatic β cells. β cells have three channels: calcium channels, sodium channels and potassium channels.

The experiment of Rosman, Braun & Zhang (2012) proved that when the number of calcium ions outside the β cell increases, it will flow into the cell through the calcium ion channel, and the intracellular potential rises to generate an action potential. Thus, beta cells are activated to regulate blood sugar by producing insulin, and can also repair pancreas damage by autoimmunity through proliferation.

Group	Estradiol (pg/ml, serum)	Osteocalcin (ng/ml)	C-Tx Terminal Telo-peptide(ng/ml)
Control Group	4.536 ± 0.343a	3.398 ± 0.294a	4.751 ± 0.244 a
Ovariectomized Rats (OVX)	3.920 ± 0.277a	4.047 ± 0.501b	5.082 ± 0.462 ab
Ovariectomized Rats Treated with SAC	4.217 ± 0.247a	3.558 ± 0.154ac	4.360 ± 0.495 a

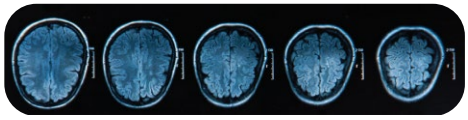
Table 18. Bone Modeling Examination Results for Five Rats with Average Standard Deviation. According to Duncan's multiple range test (Duncan's MRT), values with the different small letter superscript are significantly different among rows at $p < 0.05$. Sham: Control Group; OVX: Ovariectomized Rats; OVX + SAC: Ovariectomized Rats Treated with Sigma Antibonding Calcium Carbonate.

XIV. SAC Calcium Signals and Alzheimer's Disease

"Calcium signals play a role in communication between cells, and the synapses in the brain maintain calcium balance. If calcium signals are disturbed, brain cell degeneration will occur.

In addition, osteocalcin formed in osteoblasts has a very great effect on the cognitive ability and memory of the brain."





"About 8 years ago, I visited a Christian TV broadcasting company in Los Angeles. When I talked to the director of that company, I heard that his wife had Alzheimer's disease. I recommended taking SAC Calcium. After some time, I heard from the director that his wife had stopped taking all of the medicines used to care for her Alzheimer's, her condition improved, and she could start doing simple household tasks that previously could not be done. "

"A few years ago I met a man in his 40s. Due to work pressure, he was diagnosed with Parkinson's disease a few years ago. The tremors of the man's hands had become intense, and he could barely work outside the house. He said that less than a month after taking SAC Calcium, symptoms such as tremors of his hands had disappeared, and he could do some outside work."

1. Introduction

Cancer is the most disconcerting health disease among elderly people nowadays, followed by dementia. Dementia is the loss of cognitive functioning to such an extent that it interferes with a person's daily life and activities. It is a brain disease that is associated with memory loss and personality changes. Dementia is not only a long-term decline in memory and thinking ability, It also has a great impact on daily life, and even causes the development of symptoms that undermine human dignity.

The most common form of dementia is Alzheimer's disease, which accounts for 50% to 70% of the overall dementia, followed by vascular dementia, Lewy body dementia (LBD), which accounts for 20 to 30%, and frontotemporal dementia. The cause of dementia has not been correctly clarified, and some people think that hydrocephalus and Parkinson's disease may be the cause. In addition, chronic diseases such as hypertension, smoking, diabetes, and obesity can also become risk factors. However, the direct cause of dementia is likely to be a decrease in osteocalcin.

Although the method of treatment for dementia is not clear, for dementia that is not serious, cholinesterase inhibitors like donepezil are mainly used as therapeutic agents, but it is difficult to achieve large effects.

In 2015, about 46 million people worldwide suffered from dementia. As life expectancy increases, the onset of dementia generally occurs after the age of 60, and more than 40% of people over the age of 80 suffer from dementia. The economic loss caused by the management of dementia is approximately $6 trillion USD per year, and it is predicted to continue to increase in the future.

2. Alzheimer's Disease

According to Alzheimer's Disease International (2012), among the 291 causes per 100,000 (disability-adjusted life years (DALY)), Alzheimer's disease and other dementias accounted for 49th in 2010. In addition, from a regional perspective, the number of disability-adjusted life years for patients with Alzheimer's disease and other dementias is 19th in the high-income Pacific region, 11th in Western Europe, and 13th in Australia. North America ranks 12th, Central Europe ranks 24th, and Central and South America ranks 26th (ADI, 2012). With the increase of the human life span, the prevalence of diseases related to mitochondrial dysfunction that decline with age will increase dramatically. One of the most striking diseases is other dementia diseases, including Alzheimer's disease.

Alzheimer's disease is divided into familial Alzheimer's disease, caused by genetic variation (FAD), and sporadic Alzheimer's disease (SAD), for which the cause has not been accurately identified. Familial Alzheimer's disease accounts for about 5-10% of all Alzheimer's disease patients, most of whom are sporadic Alzheimer's disease patients (Tedd et al., 2003). The genes involved in the etiology of familial Alzheimer's disease include the PSEN1 genetic factor on chromosome 14, the APP genetic factor on chromosome 21 and the PSEN2 genetic factor on chromosome 1 (Tian et al., 2015). Mutations in any one of these three genetic factors will develop into Alzheimer's disease (Weggen & Beher, 2012)

Although the cause of sporadic Alzheimer's disease has not yet been identified, the most likely cause is the excessive deposition of the β-amyloid protein (Murphy & Levine, 2010). Beta-amyloid is a substance produced by cells in the body during normal metabolism (Wang et al., 2017). Although not a problem in other cells and tissues, this protein is harmful to nerve cells (Wang et al., 2017). The abnormal functioning of nerve cells calcium signals leads to the production of β-amyloid protein, which in turn leads to the destruction of calcium signals (Demuro, Parker & Stutzmann, 2010). Through this continuous chain process, memory loss and Alzheimer's disease progress rapidly (Demuro, Parker & Stutzmann, 2010).

3. β-amyloid and Amyloid Precursor Protein

Beta-amyloid and amyloid precursor protein are recognized as the main causes of Alzheimer's disease (Murphy & Levine, 2010). Amyloid precursor protein is an essential membrane protein for the human body, which forms the inner membrane of cells and can be found in nerve synapses (Wang et al., 2017). When nerve cells are damaged, they promote differentiation (neuronal differentiation) to play a role in repair and to help cell signal transduction and cell adhesion (Zheng & Koo, 2004). According to an experiment by Phinney et al. (1999), long-term damage to organs was found in mice with amyloid precursor protein removed, along with indications of synaptic strengthening (long-term potentiation) and the phenomenon of memory decline.

In addition, in an experiment with fruit flies, it was determined that when an excess of amyloid precursor protein is induced, the axonal transmission system is completely blocked, and the neuron cells are killed. If the amyloid precursor protein is removed, it will collapse. Aside from axonal transfer functions, facts have proven that the amyloid precursor protein should exist in an appropriate amount. (Cassar & Kretzschmar, 2016)

The amyloid precursor protein produced by the proteolysis of β-secretase and γ-secretase is β-amyloid (Storey & Cappai, 1999). β-amyloid protein exists in an aggregated form to generate oligomers (Finder & Glockshuber, 2007). Therefore, the oligomer morphology that induces the misfolding of the amyloid precursor protein causes a chain reaction similar to a prion disease, resulting in Alzheimer's disease. (Rasmussen, Jucker & Walker, 2017)

4. Calcium Signals and β-amyloid Formation

So, why is β-amyloid formed? The main reason is the destruction of the calcium signaling system (citation). The calcium signaling system is destroyed in a variety of ways, including the breakdown of the secretion system of calcium regulating hormones such as parathyroid hormone, thyroid hormone and calcitonin, which are responsible for calcium balance in the body, and the abnormal secretion of osteocalcin or renal

calcium, which is caused by reabsorption function dysfunction (Wei & Karsenty, 2015).

Once destroyed, the calcium signaling system induces the production of β-amyloid (Demuro, Parker & Stutzmann, 2010). The β-amyloid produced in this way not only induces the misfolded oligomer form of the amyloid precursor protein, induces dementia and Alzheimer's Disease, but also destroys the calcium signaling system (Demuro, Parker & Stutzmann, 2010).

β-amyloid destroys the calcium signaling system in various ways (Demuro, Parker & Stutzmann, 2010). The main mechanism is to destroy the calcium permeability of the cell membrane and destroy the calcium homeostasis of the cell (Demuro, Parker & Stutzmann, 2010). The β-amyloid protein reacts with various calcium pathways in cells (Demuro, Parker & Stutzmann, 2010). In addition to reacting with voltage-dependent calcium channels and nicotinic acetylcholine channels, it also reacts with receptors such as dopamine receptors and serotonin receptors (Kim & Rhim , 2011).

AMPA glutamate receptors and NMDA glutamate receptors are receptors that regulate the calcium permeability of the neocortex and the hippocampus, which are associated with the cognitive functions of the brain (Terry & Buccafusco, 2003), and by reacting with β-amyloid, they destroy calcium permeability and eventually cause nerve damage.

(Demuro, Parker & Stutzmann, 2010) After culturing short-term nerve cells and observing the effect of β-amyloid, it is noted that β-amyloid oligomers increase the influx of calcium through NMDA channels, and this effect brings with it an increase in reactive oxygen species and the activation of calbindin, which destroys synapses (Danysz & Parsons, 2012). In addition, continuous exposure to β-amyloid protein will reduce the expression of NMDA cells and the influx of calcium (Danysz & Parsons, 2012). One of the main sources of neuronal calcium is regulated by voltage-dependent calcium channels, but P/Q type voltage-dependent calcium channels are inhibited by β-amyloid oligomers (Nimmrich et al., 2008).

β-amyloid destroys the matrix integrity of the cell membrane (Danysz & Parsons, 2012). Beta-amyloid protein reacts with phosphatidylinositol (PI), phosphatidylglycerol (PG), and phosphatidylcholine (PC) in cell

membranes (Matsuzaki, 2007). The effect of β-amyloid was observed by fluorescence spectroscopy, and the results showed an interaction with the plasma membrane of the synaptic prototype (Plasma Membrane). Therefore, membrane fluidity, lipid environment (lipid milieu) and membrane protein proximity deformations occur, increasing calcium permeability, and thereby destroying the calcium signaling system (Müller et al., 1995).

5. Osteocalcin and Dementia

Most women experience memory loss (dementia) after menopause (Robusto-Leitao & Ferreira, 2006), which most people accept as a natural phenomenon of aging. But if this symptom is not simply memory loss, but dementia, such as Alzheimer's disease, it will be different. As mentioned earlier, an individual suffers from Alzheimer's disease due to the destruction of calcium signals or the production of β-amyloid (Danysz & Parsons, 2012). It is easy to find interesting research reports about osteocalcin accumulated in bones and memory loss and bone health (Hoang et al., 2003; Bradburn et al., 2016).

(Kersenty, 2017) A research report published by Professor Gerald Casinti from Columbia University pointed out that if the bones lack osteocalcin, memory will be impaired. This is a phenomenon that occurs when bone density decreases and osteocalcin decreases (Kersenty, 2017). In the clinical testing of mice, Professor Casinti conducted comparative experiments on the cognitive and memory abilities of mice without osteocalcin and mice with osteocalcin, and found that the memory functions of mice without osteocalcin were quite different.

This is the first time that osteocalcin has been studied in relation to memory. In addition, the clinical results boost our confidence in the prevention of memory loss and cognitive decline. If we believe the results of Professor Casinti's mouse experiments, we are able to fundamentally understand that osteocalcin can improve memory function and provide hope for the treatment of Alzheimer's disease.

CBHI has received feedback from children, students and even the elderly who take SAC Calcium to improve concentration and memory. The increase in osteocalcin that can be seen clinically in mice also supports

this view (Choi et al., 2012). In this way, the health of bones is not just a question of whether it will break or not, but the hormones produced in bones through the supply of calcium, such as osteocalcin, osteoblasts, and osteoclasts , play a role in DNA replication. In terms of cell metabolism, the metabolism of our entire body plays an indispensable role.

6. Treatment and Management of Dementia

6a. Acetylcholinesterase Inhibitors (Tacrine, Rivastigmine, Galantamine and Donepezil)

Acetylcholinesterase inhibitors are enzymes that break down the neurotransmitter acetylcholine (Moore & Hess, 1975). Acetylcholine secreted from synapses binds to acetylcholine receptors and transmits information. Acetylcholine is broken down by acetylcholinesterase, reducing the activity of cholinergic neurons (Moore & Hess, 1975). One of the representative causes of Alzheimer's disease is the action of acetylcholinesterase. Acetylcholinesterase inhibitors (cholinesterase inhibitors) prevent the action of the enzyme acetylcholinesterase, thereby reducing the decomposition of acetylcholine and preventing the necrosis of cholinergic neurons (Zhang et al., 2016). This inhibitor has a variety of ingredients, including tacrine and donepezil.

Tacrine quickly rises to the brain and has a 10-fold effect in plasma. However, due to the hydroxylation of the liver, it exhibits low life, and only 2~3% of the dosage is effective. In addition, its use has been banned due to the confirmed side effect of liver toxicity.

Donepezil, as a priority drug, is an FDA-approved drug and is used by patients with mild, moderate, and severe illnesses. Compared with tacrine, acetylcholinesterase inhibitors are more specific (Mcgleenon, Dynan & Passmore, 2001), and the effect will last for 14-21 days after taking it for an extended period of time (Bryson & Benfield, 1997). Donepezil can not be excreted normally, and it has the side effects of damaging the cytochrome p450 system and the kidneys (Mcgleenon, Dynan & Passmore, 2001).

6b. NMDA Receptor Antagonists

NMDA is composed of nerve receptors and proteins in nerve cells. It regulates cell death through direct interaction with dopamine receptors, or is responsible for signal transmission between cells. The killing of cells and the signal transmission function between cells do not affect each other, and selectively regulate and advance. Conversely, you could prevent the death of brain cells by reducing the function of cell death. Memantine is a drug developed based on this principle. As a noncompetitive NMDA receptor antagonist, it treats Alzheimer's disease by inhibiting the glutamatergic system activated by glutamate. But so far, no obvious therapeutic effect has been obtained, and side effects such as hallucinations, confusion, dizziness, headache and fatigue have been reported.

6c. Management of Patients with Dementia

The most important treatment for patients with mild dementia is family care. The more time the patient spends alone, the more rapidly their condition will deteriorate. We have experienced this all too often. However, even if the family members work together to care for severely ill patients, the burden is too great to bear. Caring for patients with dementia can also cause conflicts between family members and the breakdown of the family. This has become a disease that the government cannot solve without intervention.

In response to the increase in the incidence of dementia corresponding to the elderly population, the health care authorities of developed countries around the world have racked their brains in considering how to manage the situation, but are distressed because of the economic burden.

7. SAC Calcium Signal and ADHD, Epilepsy and Autism, etc.

"Normal calcium signals are very important for the growth and development of the fetal brain during pregnancy. In particular, sufficient calcium must be supplied during DNA replication, but if the calcium supply available is insufficient during DNA replication, genetic mutations may occur."



Figure 131. DNA Structure and Base Sequence Using a Computer Monitor

"I attended a seminar in Toronto many years ago. After the lecture, I had a conversation with a woman in her 30s. She said that she had three children, but all three of them were autistic."

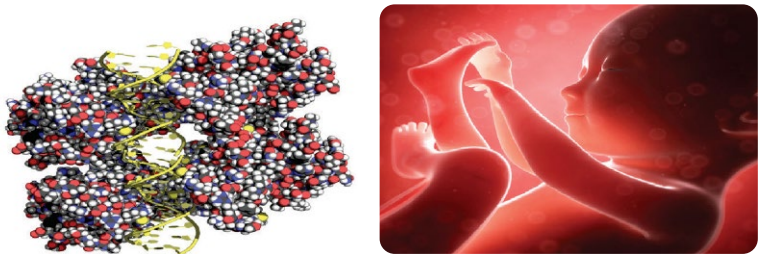


Figure 132. DNA Helix

"In order to help children with disabilities, I visited a school for children with disabilities in Seoul five years ago. We provided SAC Calcium supplements for autistic children. Many got better after taking SAC Calcium. There was one child who, until he was 11 years old, could not speak with his parents due to his disabilities. But after taking SAC Calcium, he was able to hold a short conversation with his parents. One child can now distinguish between 'hello' and 'goodbye!'. "

"A few years ago, I visited a city in the United States to participate in a seminar. At that time, I had the opportunity to meet a 60-year-old woman with a 25-year-old son. She said that her son had long experienced epilepsy, and long-term medication had no effect. Due to frequent attacks, the family members suffered from intense emotional distress that was not visible to those outside the family. I recommended to her that her son take SAC Calcium twice a day. When we contacted her again after a year and a half, after taking SAC Calcium, her son had recovered."

"A few years ago, I visited Shandong, China, to participate in a seminar. I met a woman in her 30s who had suffered from epilepsy since she was a child. She suffered from severe seizure symptoms and had seizures every day. Many times, I recommended that she take SAC Calcium. After taking it for about six months, I heard from her and she explained that she had not had any seizure symptoms for the six months after she began taking it."

In recent years, the number of children born with more than one handicap has increased significantly. According to statistics, South Korea's total population is declining and the fertility rate is also decreasing, but the fertility rate of children with disabilities is increasing.

The increase in the rate of child fertility with disabilities may be due to marriage at an older age, but this can only be confirmed through more accurate research and epidemiological investigations. In addition, the increase in the fertility rate of disabled children will not only bring with

it a great burden to the family, but also to society and to the country, so now is time to take preventive measures.

Life begins with gene replication and cell division (Rey & Prieto, 2014). Gene duplication is constantly repeated. If gene duplication is not achieved normally, mutations will occur, and the possibility of impairing brain function and organs increases (Mazouzi, Velizi & Loizoul, 2014).

The number of cells in a newborn is about 2 trillion (Sender, Fuchs & Milo, 2016). According to protein coding standards, there are 19,950 human genes (Schwarz, 2005). So, when there are so many genetic copies and repeated copies, how many normal copies can there be? Without adequate nutrient supply, gene replication cannot be achieved.

When genes are copied, calcium aids normal information transmission (Raleck al., 2017). When calcium is insufficient, normal replication cannot take place (Ralec et al., 2017) and mutation will occur.

8. Calcium Requirements During Pregnancy

Women's demand for calcium doubles after pregnancy. In other words, suppose that the usual calcium intake is 800 mg, then 1600 mg or more is required during pregnancy. This figure is also based on the supposition that pregnant women can absorb and use 100% of the calcium they ingest. Assuming that the weight of a newborn is 4 kg, the bone weight is about 800 g, and the weight of pure calcium is about 80 g. The amount of calcium a pregnant woman takes in during the 270-day pregnancy is 432 g. Assuming that the pregnant woman consumes half of that amount of calcium for herself, the fetus consumes 216 g of calcium. If you put aside the 80 g of calcium used in bones, 136 g is the amount of calcium used from the start of fertilization to delivery. In other words, the fetus consumes an average of 503 mg of calcium per day for cell division and metabolism (Miyaket al., in 2015). When the amount provided is less than this amount, the fetus will have problems in the areas of gene replication, cell division, and metabolism (Ralec et al., 2017). If there is an insufficient, continuous supply of calcium during pregnancy, it can lead to the birth of children with impairments, or to impairments during growth and development (Ralee et al., 2017). Supplementing truly effective calcium before and during pregnancy is essential for the birth of healthy babies.

Although epilepsy is a chronic neurological disorder, it is generally considered to be associated with an abnormal pituitary gland and pineal gland;

a disorder caused by the secretion of hormones. There are symptoms that are accompanied by partial or general seizures and tremors, but there are other symptoms that are not. At present, aside from the administration of hormone therapy and antiepileptic drugs, there are no other effective methods for treatment.

Over the past few years, SAC Calcium has been administered to patients with epilepsy. As a result, many patients' epilepsy symptoms have disappeared. We believe that this is because SAC Calcium regulates the secretion of hormones from the pituitary gland and the pineal gland, eliminating the symptoms of epilepsy, but this requires subsequent clinical confirmation. SAC Calcium can alleviate the symptoms associated with ADHD and autism. Especially in ADHD, the effect of SAC Calcium is most evident.

XV. SAC Calcium and Degenerative/ Rheumatoid Arthritis

"I saw a woman who has been engaged in cosmetology work for 30 years and has rheumatoid arthritis. The pain was so severe that she could not bear it without a [transdermal] patch. For two weeks, she took SAC Calcium for her pain. Symptoms disappeared, and she no longer needed other medication. In fact, her inflammation score (ESR) was reduced from 550 to 15."

"This is what happened when I was in the Canadian research institute. A woman in her 60s who lived in British Columbia visited the CBHI lab. She had suffered from rheumatoid arthritis for more than ten years. At her visit, she brought with her more than 20 kinds of health supplements that she was taking, and at the time, she was receiving gold injections that cost $1000. She said she had not seen any positive effects from any of those treatments. So, I did not hesitate to recommend taking SAC. After taking SAC for one week, she contacted me again. She said that she felt like she was in her 20s again and felt as if a weight had been removed."



Figure 133. Comparison of Hand Joints of Normal People and Hand Joints of Patients with Rheumatoid Arthritis

1. Introduction

Joint is a term used to refer to where bone meets bone. The bones are wrapped in soft cartilage, which absorbs external shocks and prevents

injuries caused by collisions between bones. As articular cartilage ages, it becomes thinner, torn, and broken due to accumulated impact and repeated use, the muscles and nerves surrounding the cartilage begin to produce inflammation. In addition, even if there is no physical shock, inflammation will occur when calcium precipitates and causes calcification. These are collectively called arthritis.

As people get older, many people suffer from arthritis, which reduces quality of life as people grow older. Arthritis is a variety of diseases that accompany joint damage. Arthritis that occurs with age is roughly divided into three types: degenerative arthritis, rheumatoid arthritis and septic arthritis, with most instances occurring in individuals from 55 to 60 years old. Arthritis primarily causes inconvenience for people. Arthritis is joint inflammation stemming from a variety of causes. The calcium in the cartilage plays a role in supporting the cartilage matrix, but if too much cartilage is used, the calcium will be consumed, calcium levels in the cartilage will decrease, and the discharged calcium will cause calcification and inflammation.

In addition, calcium deficiency or calcium excess can cause damage to the joint capsule and synovium. The joint capsule is a pocket-shaped tissue containing mucus, which plays a role in reducing joint friction in the contact between bones. The synovium is the membrane that wraps the inner surface of the joint capsule and secretes lubricating fluid. The most common arthritis symptom is joint pain or a burning sensation, but there is no effective therapeutic agent, and it is considered an incurable disease. Although there have been many therapeutic agents or health supplements that are beneficial to joints, the cartilage itself has no blood vessels, so decent effects cannot be seen. Patients can only spend every day in pain.

In order to reduce the pain of arthritis patients, doctors have prescribed many non-steroidal anti-inflammatory analgesics, but these analgesics can cause stomach bleeding, and in severe cases, liver failure, and have fatal side effects.

According to the National Centers for Disease Control (CDC), more than 7,600 patients die each year due to the side effects of non-steroidal anti-inflammatory analgesics, and more than 80,000 are hospitalized.

Repeated intake of steroids will not only cause problems such as coma,

esophagitis, peptic ulcers, and increased blood pressure, but also cause Achilles tendon ruptures, osteoporosis, bone decay and bone necrosis, and even minor shocks can cause serious fractures. Osteoporosis.

2. SAC Calcium and Degenerative Arthritis

Calcium ions are closely related to cartilage tissue. Especially in cartilage tissue, calcification can cause inflammation and become the cause of chronic diseases. Observing the organization of collagen fibers through various research papers, we can learn that with different calcium ion concentrations, tissue structure also varies.

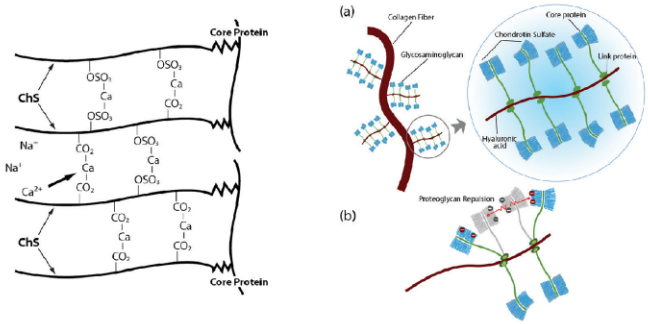


Figure 134. Cartilage Tissue Collagen Structure and Cartilage Protein Structure

The calcification of cartilage mainly includes hydroxyapatite (calcium phosphate) crystals deposited in a cell matrix, which is composed of type II collagen and cartilage proteoglycan. The calcification process in the bones and cartilage is similar. The high concentration of calcium in soft tissue is combined with the negative ions of proteoglycan. As the phosphate concentration increases rapidly, calcium ions are separated from the proteoglycan through the ion exchange effect, and its concentration is increased from calcium phosphate products to produce hydroxyl groups. Above the critical value of apatite, calcification begins. At this time, inflammation begins to appear and becomes a chronic disease, and through the non-ideal activation of immune cells, it will also develop into an autoimmune disease or rheumatoid disease.

SAC Calcium increases the ionized calcium in the blood, flows into the cartilage in a diffuse form, and supplies calcium to the collagen protein. As a result, cartilage tissue is regenerated, mesenchymal stem cells are activated, and cartilage cells are proliferated. In addition, the hydroxyapatite calcified by phosphate in an acid environment gradually dissolves the apatite calcified due to the increase in pH as calcium ions increase.

XVI. SAC Calcium and Viral Diseases (Viruses)

"With the exception of bone marrow transplantation, there is no other treatment for aplastic anemia. I met a student in his second-year of university who suffered from this illness. I suggested that he take SAC Calcium, and I heard back from him that his health had greatly improved after taking SAC Calcium. After recovering from the illness, that student who took SAC graduated from university. He now works in a large general hospital in Gangnam, Seoul. While working at the hospital, he encountered an outbreak of Middle East Respiratory Syndrome (MERS) back in 2015, shortly after employment. While many patients and doctors in the hospital were infected with and prone to infection from MERS, he was one of the few doctors that did not test positive for MERS. A mass infection incident occurred due to exposure to the virus. But surprisingly, this young doctor was not infected. The difference between the other staff and him was that he had been taking SAC Calcium."

1. Introduction

Viruses begin with ribonucleic acid genetic material. The ribonucleic acid is centered on phosphoric acid and is formed by the combination of ribose and nucleic acid . A virus forms a full-form particle on the outer wall of the cell in the state of sodium, which is called the virus body (virion) or virus particle.

The proliferation of the virus centers on phosphoric acid and proliferates in the form of a copolymerization of ribose and nucleic acid. The virus cannot produce the energy required for metabolism or synthesize proteins, so the existing protein is used to make capsids and multiply. When multiplying, the virus circulates and multiplies in the extracellular fluid, which must meet the absolute conditions of the body fluid environment. The environmental conditions for growth are abundant phosphoric

acid and a low temperature (below 36°C).

If this condition is not created, the virus will not multiply. This is the function of calcium ions. Calcium ions play an absolute role in the sugar + oxygen reaction in the mitochondria, contribute to the rise of body temperature, and also inactivate the RNA.

In Europe, in the 18th century, cold patients were injected with calcium. Due to the coagulation caused by the binding of calcium and plasma proteins, etc., which caused the clogging of blood vessels, its use was discontinued. Humans seem to have known that calcium works on viruses for a long time now.

2. The Inactivation Effect of SAC Calcium on Viruses

SAC Calcium can increase the concentration of ionized calcium in the blood, increase the concentration of calcium in the intracellular and external fluids, combine calcium ions with phosphoric acid, and promote RNA inactivation. 5 mg of SAC Calcium can instantly supply 1.25×10^{19} calcium ions to the blood and cells. Of course, when an infection is severe, calcium needs to be continuously supplied every three hours.

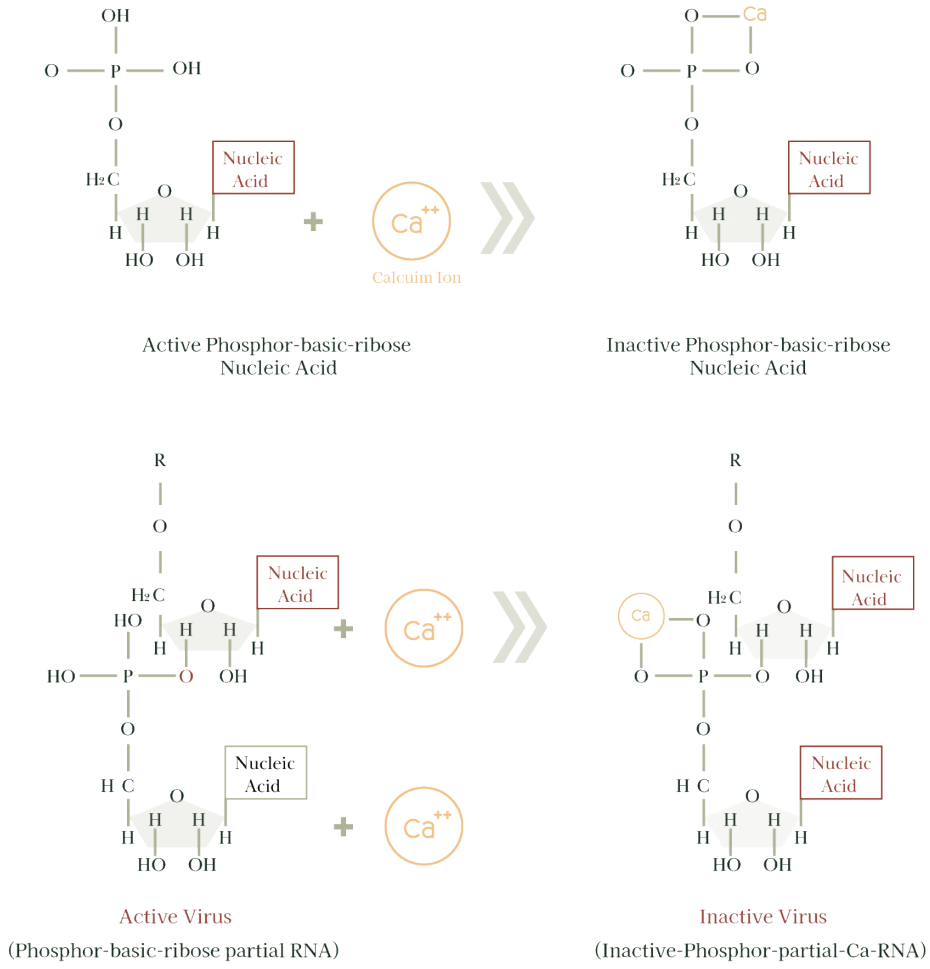


Figure 135. Inactivate and Active Viruses and Calcium ions

The HIV virus is no exception. The HIV virus will be eliminated when the volume of ionized calcium in the blood remains high. Of course, due to the long incubation period, it is impossible to conclude whether it is eliminated or inactivated. However, several clinical results have been performed on the HIV virus and significant effects have been confirmed.





Mikey, 71 years old (as of 2012), Vancouver, Canada	
Date	HIV-1RNA (Viral Load) (Copies/ml)
2011. 11. 20	2300
2012. 02. 13	50





Duke, 6 years old (as of 2012), Ghana *Congenital HIV carrier	
Date	HIV-1RNA (Viral Load) (Copies/ml)
2012. 01. 13	2450
2012. 01. 20	20





Patrick, 65 years old (as of 2012), Tanzania	
Date	HIV-1RNA (Viral Load) (Copies/ml)
2012. 06. 02	2380
2012. 06. 08	75

Figure 136. Based on these remarkable curative effects, it is expected that SAC Calcium will be able to achieve use in detailed clinical trials for the management of HIV carriers and AIDS in the future.

"I visited Moshi, Tanzania, in November 2012. I met a nurse then and heard from her that he is still alive and very well. He had been bedridden before taking SAC Calcium"



Figure 137. HIV Carrier Children HIV in Chiang mai,Thailand

3. SAC Calcium Trial on COVID-19

This case study was conducted to evaluate the effect of SAC Calcium as SAC Therapy for active COVID-19 in humans from March 28 to April 28, 2020. Zhongnan Hospital of Wuhan University, Wuhan, China.

In hematological analyses, there was a significant increase in all metrics and all diagnoses reported negative in every active COVID-19 patient treated with SAC, as follows:



ZHONGNAN HOSPITAL OF WUHAN UNIVERSITY
CENTRAL LABORATORY

Sample Test Data Report For COVID-19 Treatments
Date: 1st, 28, 2020
Number of People Tested: 7 People (5 Males and 2 Females)

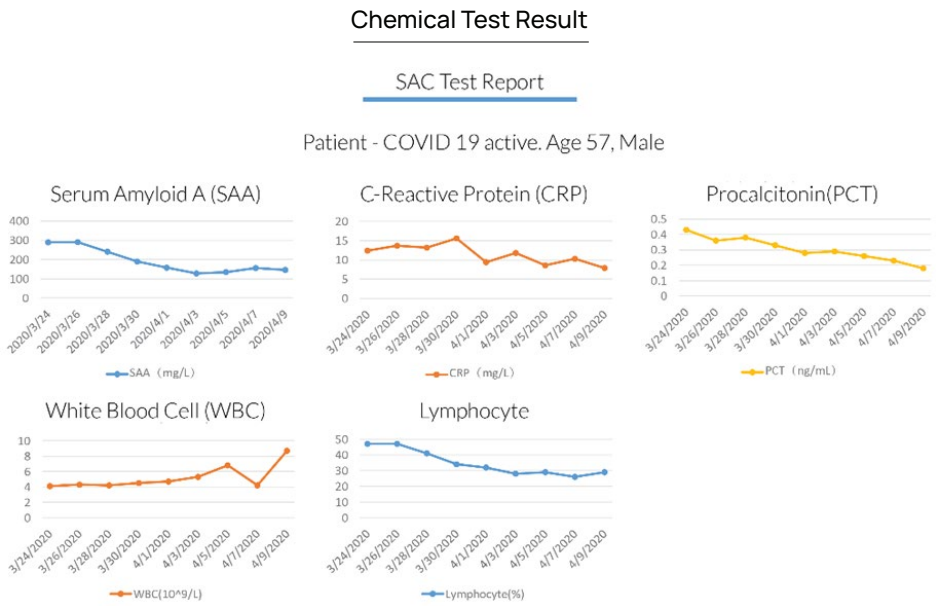


Figure 138. Nucleic Acid Test Results were Positive and Diagnosed with COVID-19 on Mar. 22, 2020. By Wuhan Univ. Zhongnan Hospital. Central Lab. Product Name: SAC (Made in Canada). Nucleic Acid Test Results were Negative (Apr. 13, 2020).

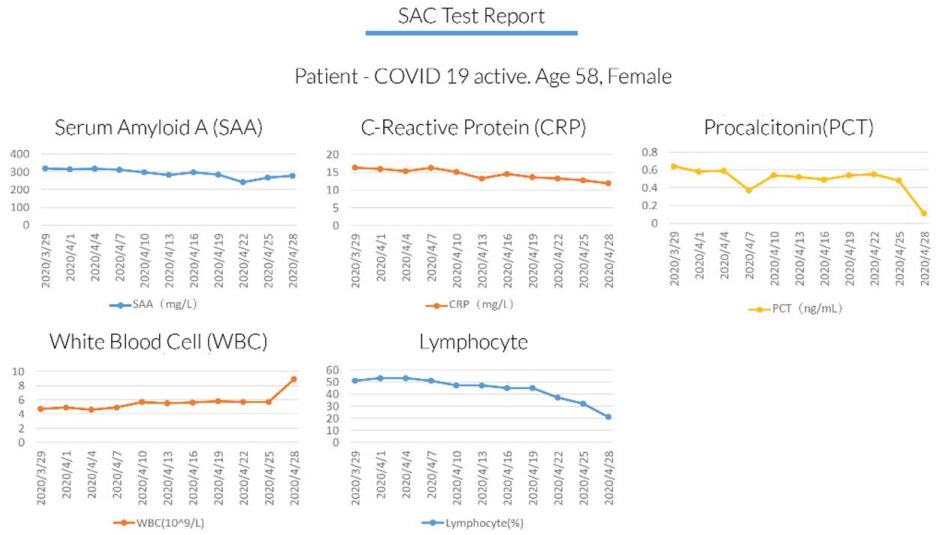


Figure 139. Nucleic Acid Test Results were Positive and Diagnosed with COVID-19 on Mar. 27, 2020. By Wuhan Univ. Zhongnan Hospital. Central Lab. Product Name: SAC (Made in Canada). Nucleic Acid Test Results were Negative (Apr. 25, 2020).

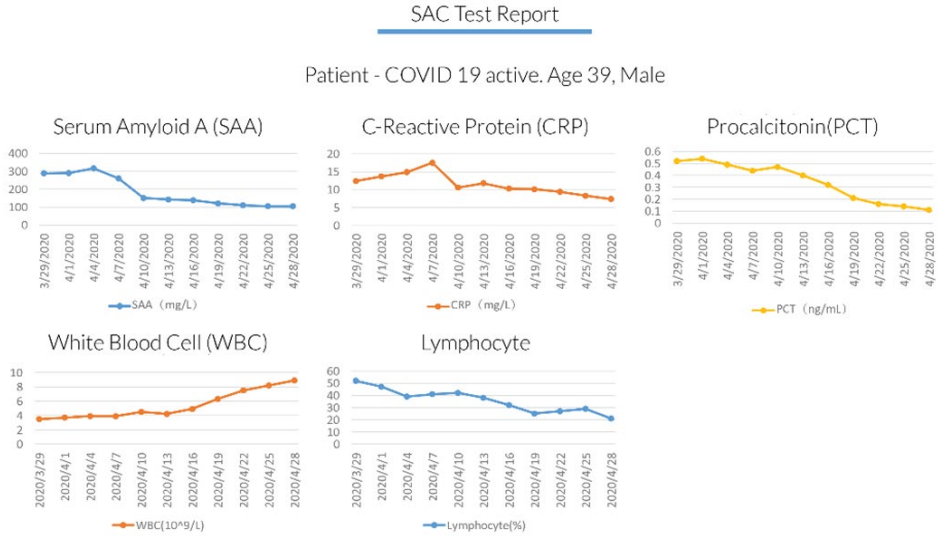


Figure 140. Nucleic Acid Test Results were Positive and Diagnosed with COVID-19 on Mar. 28, 2020. By Wuhan Univ. Zhongnan Hospital. Central Lab. Product Name: SAC (Made in Canada). Nucleic Acid Test Results were Negative (Apr. 19, 2020).

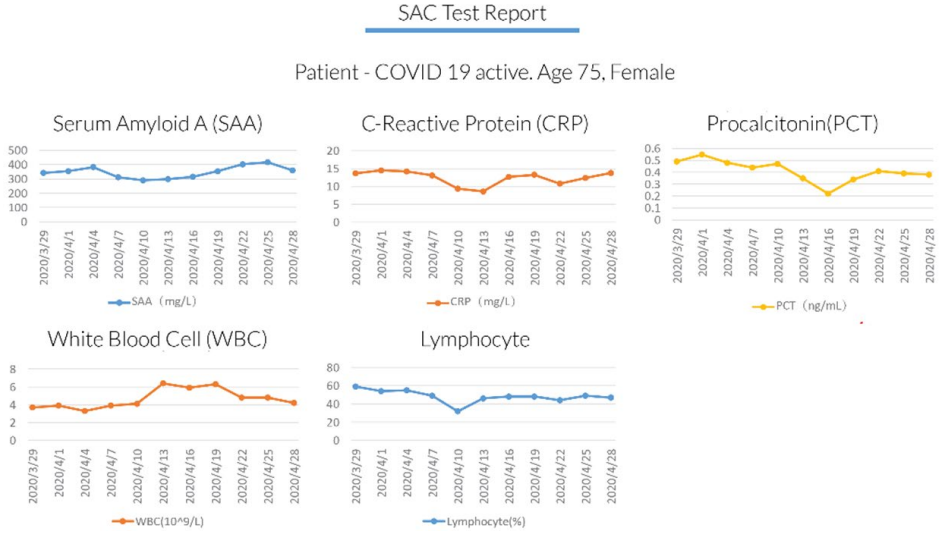


Figure 141. Nucleic Acid Test Results were Positive and Diagnosed with COVID-19 on Mar. 28, 2020. By Wuhan Univ. Zhongnan Hospital. Central Lab. Product Name: SAC (Made in Canada). Nucleic Acid Test Results were Negative (Apr. 28, 2020).

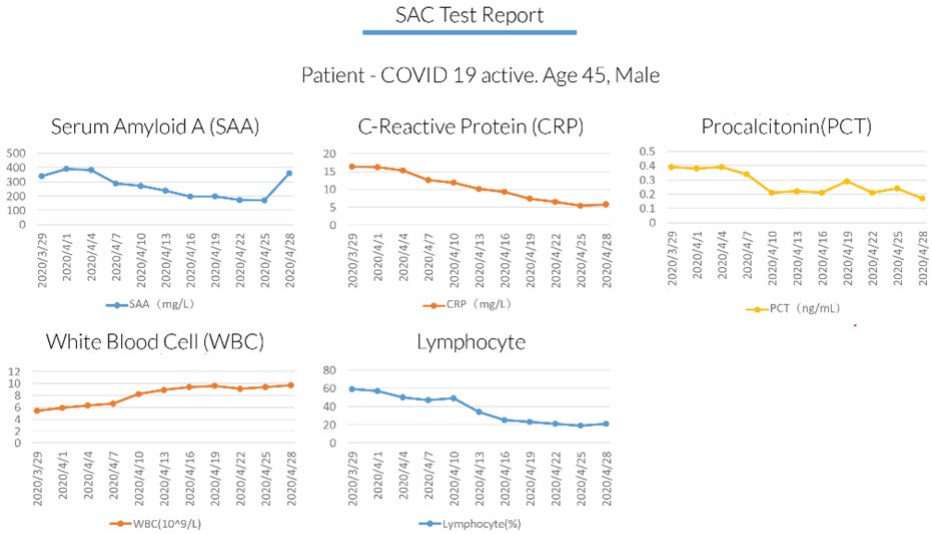


Figure 142. Nucleic Acid Test Results were Positive and Diagnosed with COVID-19 on Mar. 28, 2020. By Wuhan Univ. Zhongnan Hospital. Central Lab. Product Name: SAC (Made in Canada). Nucleic Acid Test Results were Negative (Apr. 25, 2020).

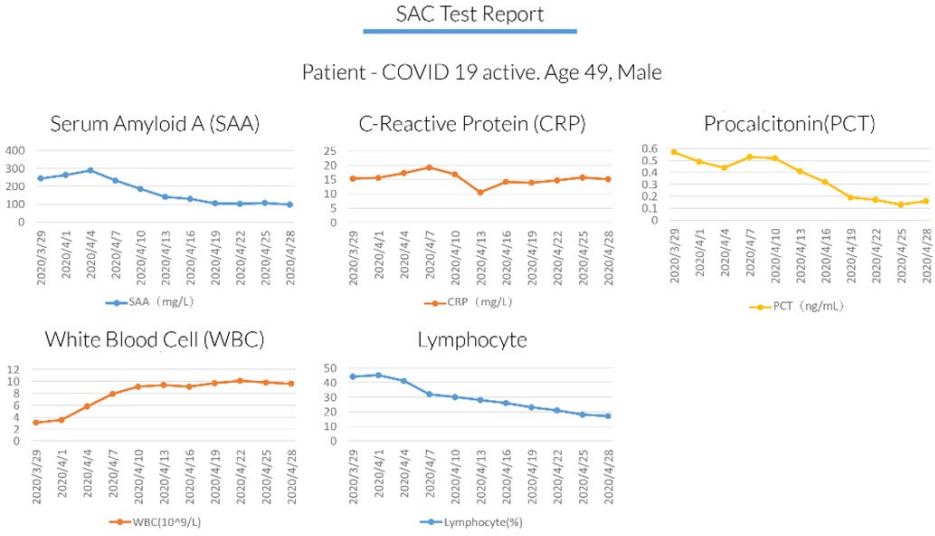


Figure 143. Nucleic Acid Test Results were Positive and Diagnosed with COVID-19 on Mar. 28, 2020. By Wuhan Univ. Zhongnan Hospital. Central Lab. Product Name: SAC (Made in Canada). Nucleic Acid Test Results were Negative (Apr. 16, 2020).

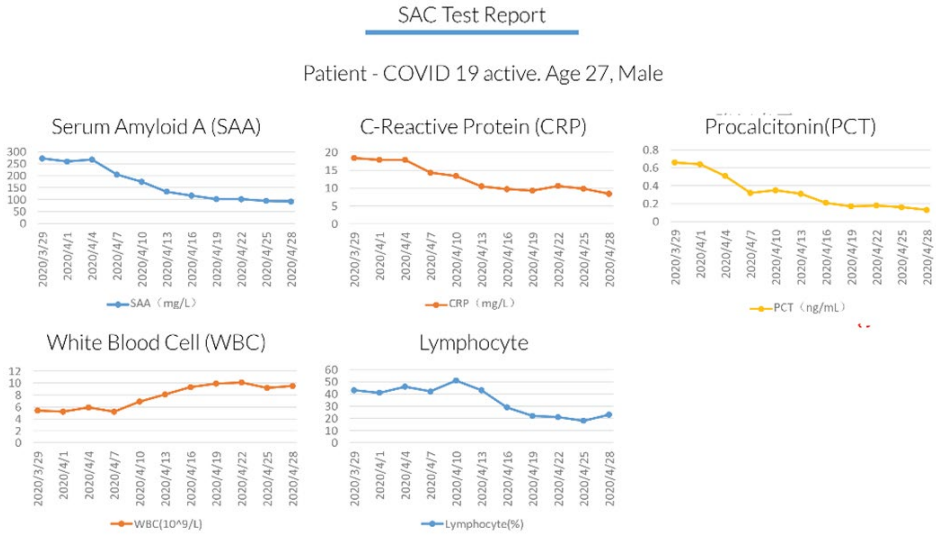


Figure 144. Nucleic Acid Test Results were Positive and Diagnosed with COVID-19 on Mar. 28, 2020. By Wuhan Univ. Zhongnan Hospital. Central Lab. Product Name: SAC (Made in Canada). Nucleic Acid Test Results were Negative (Apr. 28, 2020).

XVII. Sigma Anti-bonding Calcium Carbonate (SAC) Enhances the Wound Healing Process

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2 College of Pharmacy and Institute of Pharmaceutical Sciences and Technology, Hanyang University, Ansan, Republic of Korea
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4 CBHI (Calcium & Bone Health Institute, Canada), Coquitlam, British Columbia, Canada

1. Introduction

Sigma Anti-bonding Calcium (SAC) is calcium carbonate produced in a unique process using oyster shells as essential ingredients. Calcium carbonate has a Sigma Anti-bonding Molecule with weak binding force. This property allows molecules to quickly dissolve and be absorbed easily into cells. It can influence environmental calcium levels and modulate calcium homeostasis in vivo.

SAC produces active calcium during the body's metabolism and helps protein-binding calcium ionize. Calcium in serum exists in three forms: 40% calcium-binding protein, 10% inorganic calcium, and 50% ionized calcium. Only ionized calcium is a physiologically active form. Wound healing is a calcium-mediated process. After cutaneous injury, a cascade of events occurs resulting in tissue repair. Tissue repair is classically divided into an inflammatory phase, a proliferation phase, and a remodeling phase. The inflammatory phase starts immediately after the wound, and the inflammatory cells act to remove bacteria and foreign substances that have entered when the wound occurs and then prepare for smooth tissue production during the proliferative phase.

The proliferative phase is a recovering wound through fibroblast migration, collagen synthesis, angiogenesis and granulation, and tissue epithelialization. The remodeling phase is the stage in which newly created tissues gain strength and flexibility. The collagen fibers are reconstructed,

and the tissue is remodeled and matured, thereby increasing the tensile strength. A plethora of cells, enzymes, cytokines, hormones, and ions are involved in this process.

Metal ions such as calcium, zinc, and magnesium are involved in wound healing, One of the most important ions is calcium. Calcium is a key secondary messenger that is involved in several signaling cascades critical to wound healing. Calcium influx into cells is known to regulate inflammatory cell infiltration, fibroblast proliferation, and keratinocyte migration. Furthermore, calcium has an established role in the normal homeostasis of skin and is a modulator of keratinocyte proliferation and differentiation. These features are highly relevant to the skin, which exists in a perpetual state of dynamic equilibrium with its environment. Collectively, we hypothesized that the topical treatment of SAC cream enhances wound healing

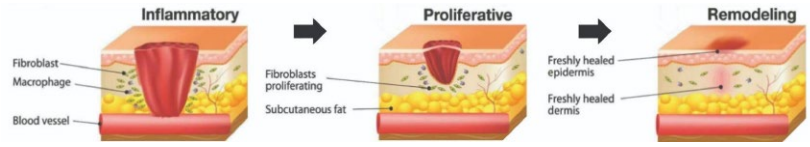


Figure 145. Skin inflammatory and remodeling process.

2. Methods

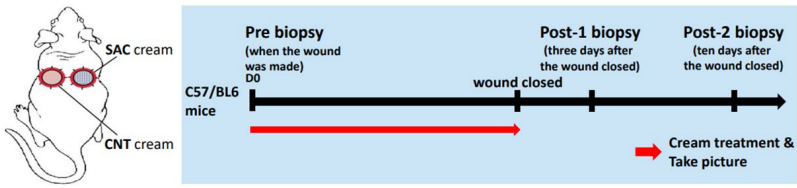


Figure 146. Generation of Wound and Time-line of the Experiment

The effectiveness of SAC on wounds was confirmed using a 9-month-old female C57/BL6 mouse. The mice were shaved using clippers, and each day their hair was trimmed using scissors. The mice were anesthetized using vaporized isoflurane. We made a complete excisional wound model using 5 mm punch biopsy, as shown in Fig. 1, and biopsies were collected

on both sides of each mouse's back as Pre-biopsy samples.

After the procedure, CNT cream was applied to the left-wound and SAC cream was applied to the right wound twice a day. Photographs were taken every day from immediately after the wound was made until it closed, and the size of the wound was measured using CellSens imaging software (Olympus). The wound size of Day0 in each mouse was set to 100%, and the measured wound size was expressed against Day0 as a percent reduction. Each mouse was housed in a single cage until the study was completed.

3 days after the wound closed, a post-1 biopsy was taken and 10 days after it closed, a post-2 biopsy was taken. After collecting the biopsy, each biopsy was fixed in 4% paraformaldehyde and stained with hematoxylin and eosin (H&E). The stained cross-section was further digitally imaged using Zeiss AX.IO Imager M2 microscope (Zeiss, Nashville, TN) at 20x magnifications, and epidermal thickness was measured as shown in Fig. 3. As for the epidermal thickness, the ETI (Epidermal Thickness Index) was calculated by measuring the positions of five different locations in the biopsy sample and obtaining an average value.

3. Results

3a. SAC improves wound healing rate

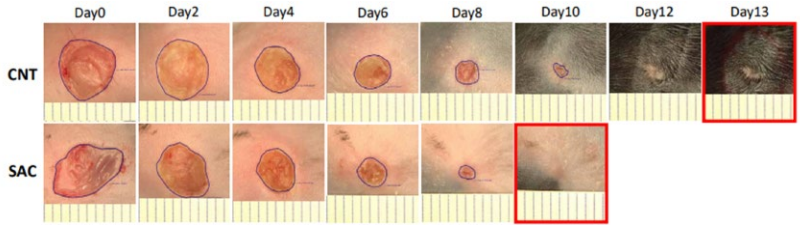


Figure 147. When the SAC was topically applied to the wound area, it was observed that the wound healing rate was improved compared to the CNT cream.

These are the representative pictures of the wound from day 0 to day 13, red boxes represent the closed images, the blue shapes were drawn along the perimeter of the wound to measure a wound size in CellSens software.

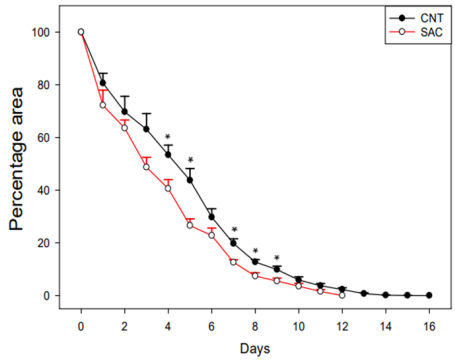
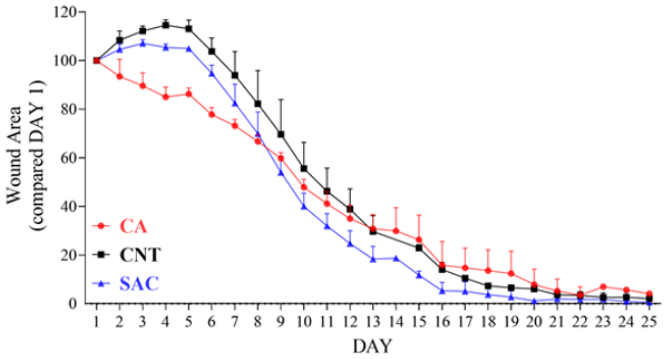


Figure 150. Wound healing of SAC Cream and control



* CA: Calcium alginate

Figure 151. The effectiveness of applied SAC cream on wound closure in diabetic mice

The graph shows means and standard errors of wound size change at each day from wounds created (Control cream and SAC). The wound size of Day 0 in each mouse was set to 100%, and the measured wound size was calculated against Day 0 as a percent reduction. * denotes $P < 0.05$.

3b. SAC improves wound healing while increasing epidermal thickness

Biopsies were taken at Pre biopsy (when the wound was made), Post-1 biopsy (three days after the wound closed), and Post-2 biopsy (ten days after the wound closed) to measure the epidermal thickness. No cream

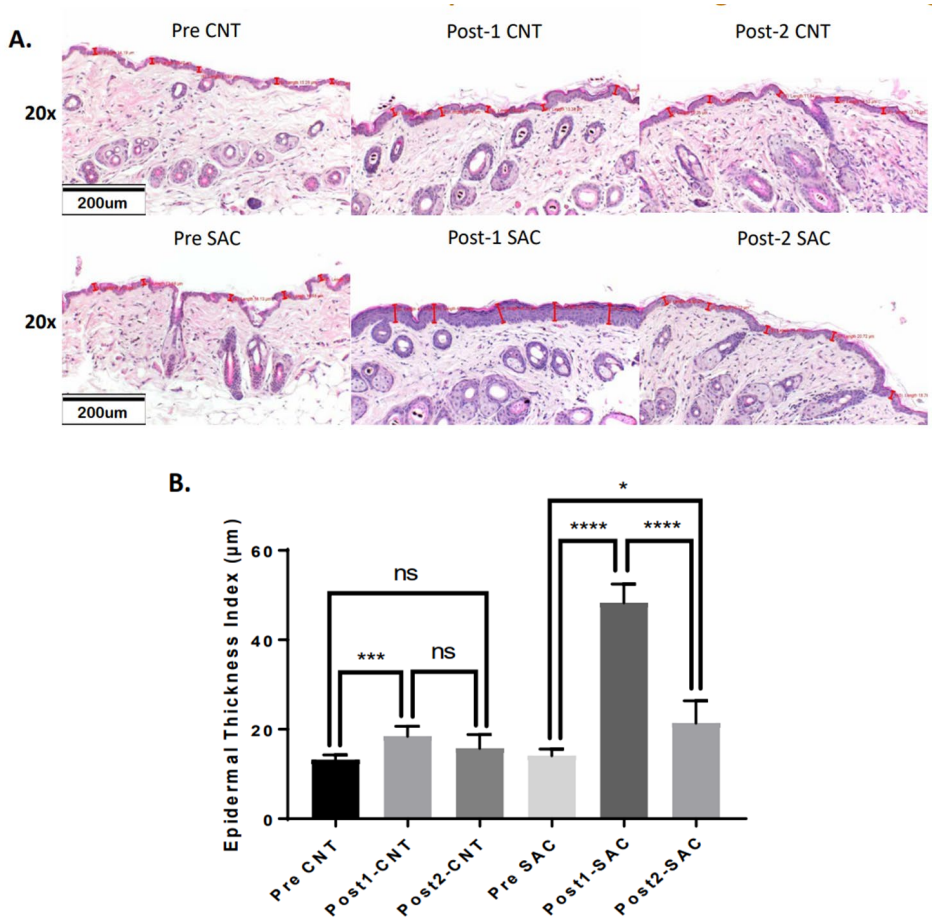


Figure 152. Figure SAC increases in epidermal thickness during the wound healing

A. Each biopsy was fixed in 4% paraformaldehyde and stained with H&E. The stained cross-section was further digitally imaged using Zeiss AX-.IO Imager M2 microscope (Zeiss, Nashville, TN) at 20x magnifications and epidermal thickness were measured.

B. The ETI (Epidermal Thickness Index) was calculated by measuring the positions of five different locations in the biopsy and obtaining an average value. - ns denotes non significant;

* denotes $P < 0.05$; *** denotes $P < 0.001$; **** denotes $P < 0.000$

3c. SAC improves fibroblast proliferation without cytotoxicity

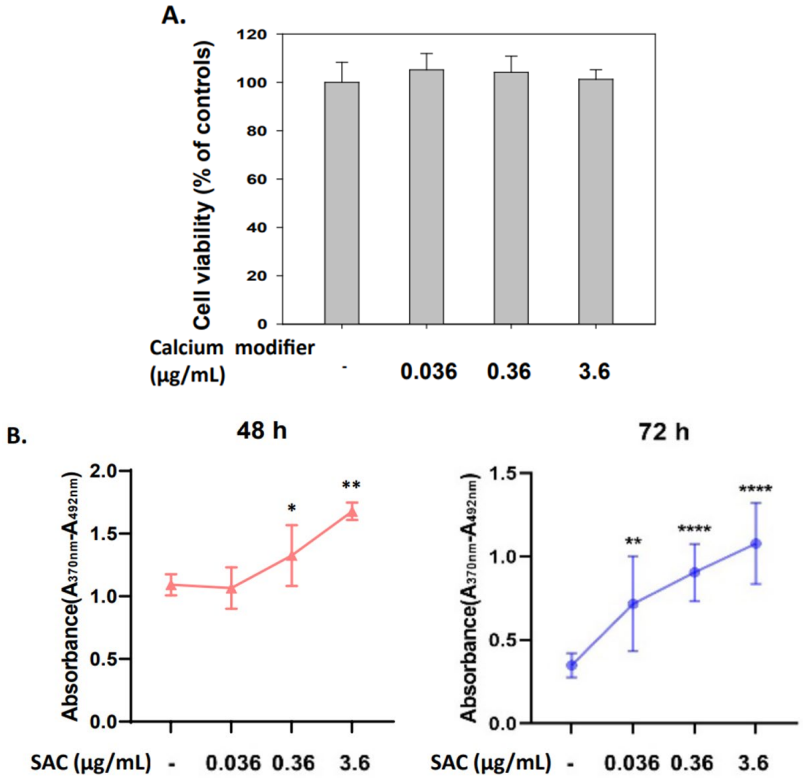


Figure 153. Figure SAC is not cytotoxic and improves HDFa proliferation

A. Cytotoxicity was tested for adult Human Dermal Fibroblast (HDFa) cells using the CCK-8 assay. The SAC solution was diluted in ultrapure water and treated at each indicated concentration.

B. BrdU analysis (Roche) was performed to study the effects of SAC on HDFa proliferation. 2E3 or 1E3 cells for 48 or 72 hours were seeded onto 96- well plates. The given SAC concentrations were treated for indicated time periods.

* denotes $P < 0.05$; ** denotes $P < 0.01$; **** denotes $P < 0.0001$

3d. Conclusion

Sigma Anti-bonding Molecule Calcium Carbonate (SAC) is a physio-chemically modified calcium carbonate with a unique weak bonding structure that significantly increases the calcium absorbing rate in our body. In this study, we found that SAC improves wound healing via increasing epidermal thickness. It is at least in part, contributed by improvement of a skin fibroblast proliferation. SAC can be used to treat skin damage. Further studies are currently conducted to determine through which process SAC causes changes in skin tissue.

3e. Acknowledgements

This research was supported by the Calcium and Bone Health Institute (CBHI) under award no. CBHI CRA C23992 and Mr. Song was supported by Korea Health Industry Development Institute under the Biomedical Global Talent Nurturing Program. All procedures related to animal work have been approved by the UAMS Institutional Animal Care and Use Committee (AUP 4151).

XVIII. Emotions and Calcium Signals From SAC Calcium

Calcium is not only for the bones. It also plays an important role in human emotions. How can calcium help in controlling emotions?

"A woman in her late forties had received counseling for severe depression. She had married early, her children were in university, and she was living an affluent life, but she suffered from depression. She was taking antidepressant drugs, and many days and nights she would stay up all night staring into the void. Whenever she drove on the bridge, she was afraid she might drive into the water, so she avoided driving her car. One day, she asked me for help. I'm not a psychiatrist, so I asked her how she found me. She replied, "I watched a YouTube video and listened to a lecture on calcium's role in controlling brain hormones." I let her take some SAC Calcium, and the next day she called me and said she slept very well and thanked me because she felt refreshed."

"A 17-year-old autistic teenager who had had no conversation with his mother wrote a message indicating that he had experienced a big life change saying, "I noticed and commented on my mom's hair when she came home after getting her hair permed."

"There was a first-grade elementary school student with ADHD, who had never been able to sit at his desk for at least five minutes. After taking SAC Calcium, he is able to read books for more than half an hour and does not want to go out to play."

"A woman in her fifties who had been confined to her room because of a panic disorder has taken SAC Calcium and made cookies and brought them to share with me."

We experience a variety of emotions every day, and these emotions are transient. But when one of these emotions becomes dominant, it can start to affect your behavior negatively. Did you know calcium signaling plays

a vital role in controlling your emotions? Emotions are mediated by neurotransmitters like dopamine, norepinephrine (or noradrenaline), and serotonin, which are secreted or inhibited by the regulation of calcium signals in the nervous systems. When these neuropeptides are excessively or inadequately secreted, or when the proportions are constantly unstable, our emotions become unstable.

1. Dopamine

Dopamine is a neuropeptide that is involved in various physiological functions, such as focus, attention, memory, desire, happiness, and vigilance. When the level of dopamine is excessively high, it can lead to ADHD, control disorders, mental disorders, etc. On the contrary, when there are dopamine deficiencies, it can result in Parkinson's disease. Dopamine is heavily linked to reward-seeking behaviors.

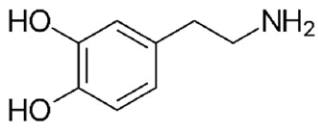


Figure 154. Chemical structure of dopamine

2. Norepinephrine

Excessive norepinephrine may cause anxiety, negative feelings, and stress response.

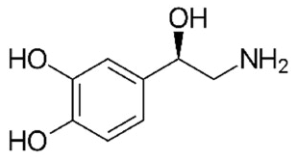


Figure 155. Chemical structure of norepinephrine

3. Serotonin

Serotonin suppresses dopamine and noradrenaline. Serotonin helps people to calm down and prevents being overly excited or having anxious feelings. However, high levels of serotonin may cause depression, sad moods, and helplessness, incompetence, isolation, vanity, insomnia, weight loss, loss of appetite and libido, loss of enjoyment and lose interest in daily life.

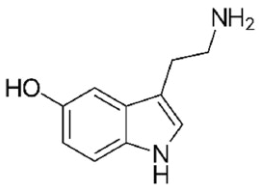


Figure 156. Chemical structure of serotonin

Therefore, emotions are formed by these neuropeptides interacting with each other, and people with an adequate presence of serotonin tend to have a better quality of life, although there might be other factors that affect the quality of life. There are numerous treatment options for mental disorders. However, many of them have side effects associated with long-term use.

Through countless research papers and case studies, we have come to the realization that emotions are controlled by numerous neuropeptides, and these are directly related to calcium signals. There is no doubt that the role of calcium in human emotion is crucial. Mental health disorders are one of the leading contributors to the global burden of disease, and it affects not only an individual's emotional health but also their quality of life. Numerous factors cause mental disorders, including age, dietary factors, alcohol consumption patterns, genetics, and so on. A cancer diagnosis may have huge psychological and physiological impacts on most patients and those around them. Depression, anxiety, and fear are probably the most frequently experienced responses to a cancer diagnosis. Cancer-mediated mental disorders are not only caused by the fear of death, suffering, pain, or all the unknown consequences that lie ahead by cancer, but physical symptoms like pain, fatigue, nausea, or changes in

body image may cause emotional distress as well. If cancer therapies neglect the significance of mental disorders in cancer progression, it could severely affect patients' emotional health and, thus, the overall outcome of therapy. For instance, a patient may reach the point where he/she feels that they are worthless and hopeless and lose the willingness to cooperate in cancer treatments. More seriously, severe emotional distress may worsen quality of life and may put patients at risk for suicide. Therefore, spiritual and emotional healing during cancer treatment is essential to encouraging quality recovery and to make them feel that every individual is deserving of life. Therefore, it is evident that calcium intake plays an important role in the alleviation of depressive symptoms and depression in cancer patients.

4. So, what type of calcium can control unstable emotions?

Only physiological ionic calcium can control emotions.

First of all, bones are the source of calcium, and their density is an important indicator of bone health. Let's talk about bone density and emotions. As they grow older, more and more people worry about their bone health. They search out calcium-rich food, such as anchovies, milk, cheese, etc. However, it is difficult to raise your bone density via food sources. Why doesn't your bone density increase? Why doesn't your bone density increase when you take 1000 mg of calcium supplements every day for several years? Why can't osteoporosis drugs with bisphosphonate, estrogen, and parathyroid hormone derivatives increase bone density?

Let's find out how SAC Calcium helps people who have recovered from depression, panic disorders, ADHD, Parkinson's disease, schizophrenia, autism, etc. Unlike regular calcium, SAC Calcium is easily absorbed into the body as ionic calcium without the aid of vitamin D.

XIX. Conclusion

By analyzing the current trend of treatments for aging-related chronic degenerative diseases including osteoporosis and cancer, it became clear that all these diseases are related to a dysfunction in calcium influx and efflux. As the flow of ionized calcium is altered, it is calcified in the mitochondrial matrix, which results in secretion of reactive oxygen species (ROS). Moreover, as the calcium homeostasis system is deconstructed, bone mineral density (BMD) and the bone turnover rate are altered, which ultimately causes the deterioration of bone health.

Through our research, we have found that SAC can be a practical treatment option for treating chronic degenerative diseases, as it balances calcium homeostasis and initiates calcium signaling at both of the extracellular and intracellular levels. As Choi et al. (2011) have investigated, SAC has a notable efficacy in elevating BMD as they have proven with their animal experiment. As their BMD rate increased greatly when ovariectomized rats were treated with SAC for 12 weeks, it became clear that SAC is useful as a treatment for osteoporosis. Moreover, SAC is also used to treat chronic neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Parkinson's disease and Alzheimer's disease. As calcium modifies mitochondrial function by balancing intracellular calcium levels, it can decrease ROS production.

Moreover, it has been proven that SAC helps to initiate the activity of mesenchymal stem cells (MSCs) by regenerating mitochondrial function. As more ATP is produced and MSC activity commences via the depolarization caused by the influx of calcium ions, it can stimulate the cell division of MSCs. As MSC activity is initiated, this can be the key to treating chronic diseases. For example, as MSC activity in bone marrow commences, mesenchymal stem cells can transform to chondrocytes, which can help to manage osteogenic arthritis.

Furthermore, as calcium enhances the activity of mesenchymal stem cells, it helps to develop dental bone health, which is helpful to rebuilding dental bone after dental implants. Moreover, calcium deficiencies and unbalanced levels of ionic calcium can contribute to the risk of developing dental gum and periodontal diseases. Therefore, as the efficacy of

SAC has been proven to balance extracellular and intracellular calcium levels, the intake of SAC Calcium is recommended to prevent the growth of bacteria in the gums and improve gingivitis and periodontal health.

Also, as SAC modifies mitochondrial function, it can adjust protein synthesis, which helps to produce p53 protein and POT1. Also, by secreting apoptosomes such as cytochrome c, it can help to effectively target the cancer cells when it is introduced into the cell. Moreover, as SAC neutralizes pH levels by forming calcium lactate and calcium ammoniate, it can prevent cancer cell proliferation. Furthermore, as calcium reduces TXNIP protein expression by inhibiting the production of glutamine, SAC can be a treatment for cancer.

Case studies have shown that SAC has notable efficacy in treating cancer. Ms. Holt and Mr. Kim's data demonstrate the likely effects of SAC. As they completely recovered from cancer by taking SAC, it is hard to deny the efficacy of SAC while the clinical data provides proof of its effects. Moreover, as their hematological data also significantly improved, it would be a wise idea to try SAC as a cancer treatment option. Currently used cancer treatments have many adverse side effects, therefore, it is essential to find an alternative option that can enhance overall body metabolism.

Through a thoughtful, systematic literature review, it became more evident that SAC can be an innovative approach to treating chronic degenerative diseases. Even though there has been limited research conducted to date around the efficacy of SAC, it is crucial to conduct further investigation into the effects of SAC as significant improvements have been seen when applying it to patients with chronic degenerative diseases, such as osteoporosis and cancer.

XX. SAC Columns

1. Osteoporosis in Old Age





Exactly how much do we know about calcium, one of the most well-known minerals?

Today, I want to discuss common knowledge that many people are either unaware of or misunderstand.

Calcium and Health

Many people think calcium exists only in the bones and joints. However, calcium is a crucial factor that helps to maintain DNA information and body temperature, myocardial contraction and relaxation. When there is not enough calcium in our body, more than 150 diseases, including hypertension, diabetes and cancer, could occur.

If we include diseases that are indirectly caused by calcium deficiency, we can say that all the diseases in our body are caused by a calcium deficiency. In particular, women have a sharp decline in bone density after menopause, which increases the risk of osteoporosis. To compensate for this, they may consume calcium supplements or calcium-rich foods, or take osteoporosis or osteopenia treatments. However, despite the intense utilization of numerous treatments and calcium agents, the osteoporosis population is on the rise, and so, economic losses are increasing exponentially.

According to the results of the study, in the 1990s, one out of three women in their 50s and one out of five men had osteoporosis, but in 2010, one out of 2.5 women and one out of four men made up the osteoporosis

population.

As of 2010, the Canadian government spends more than $4 billion annually to cover treatments for fractures caused by osteoporosis, and the US government spends tens of billions of dollars.

So why does an increased intake of calcium not increase bone density?

There are two types of calcium in the blood: one is ionic calcium and the other is the protein-bound form of calcium.

As the concentration of plasma ionized calcium increases, hormones are released from the thyroid gland, activating osteoblasts that make bones.

But the calcium we ingest is the protein-bound form of calcium in the blood, so it does not stimulate the thyroid.

Consumed protein-bound calcium can not be converted into ionic calcium due to poor eating habits, lack of exercise, and weakened stomach acids caused by aging.

Excessive calcium absorbed into the blood is accumulated in the blood vessels, causing calcification, arteriosclerosis, or heart attack.

The fact that calcium consumed for the good of your body can cause illness will surprise many people.

Osteoporosis and Diagnosis

When we get older, the amount of calcium consumed is less than the amount of calcium expelled from the bones.

Calcium is not absorbed well by bones, so the amount of bone is reduced and the density is lowered. This is called osteoporosis. The most common diagnostic method for measuring bone density is the use of X-rays (DEXA).

This is accurate in diagnosing the bone density of the vertebrae, femur and wrist. There is no pain involved in this procedure.

The bones are partially dense, depending on where they measure, so they T-score is evaluated using the mean values of the waist (lumbar) and femoral region.

The T-score value is a numerical reduction value comparing the maximum bone mass of a person. If a 10% loss is lost, then -1.0; if 20% is lost, then -2.0.

This T-score value can be seen as normal up to -0.9, osteopenia up to -1.0 ~ - 2.4, and osteoporosis from -2.5.

Calcium Supplements

We can see that various types of calcium supplements are being sold, claiming high quality calcium levels and absorption rates.

These products are absorbed only by taking vitamin D, but the amount of absorption is very minimal, and the absorbed calcium is not used properly.

Many people still take these calcium supplements, but the reason for the lack of improvement in bone density is because they are absorbed into the blood as protein-bound calcium, as mentioned earlier, and if they are not converted into ionic calcium, they can not activate osteoblasts.

Excessive intake can lead to diseases such as arteriosclerosis, heart failure, and stroke due to calcification.

Most of the osteoporosis treatments developed to date are based on bisphosphonate. It inactivates osteoclasts and prevents calcium from escaping from the bone. Although bone density appears to have risen, the density of bone can not be said to have increased in a healthy manner because dead cells are also bound together to increase the density.

This method coats the bones to prevent ionic calcium from further escaping from the bones, which can lead to greater risk in the event of an emergency. The bones store calcium, just as banks store their customers' money, and when there is a lack of ionic calcium in the body, calcium is drained from the bones.

If an emergency such as a heart attack occurs, the necessary calcium is generally supplied by removing it from the bones because the heart can not perform without calcium. But when the bones are coated and calcium can not be extracted from the bones when there is an emergent need, the situation will eventually become more serious. In addition, there are serious side effects that occur, such as fractures, due to the hardening of the bone when taking supplements long-term. Even doctors are aware that these treatments do not offer much help in improving osteoporosis.

SAC (Sigma Anti-bonding Calcium) Introduction

CBHI's SAC calcium is the first to directly transfer ionic calcium to the blood in the body and activate the conversion of protein-bound calcium into ionic calcium. General calcium is first needed to bind vitamin D and the protein to be absorbed, and is present in the body in the form of protein-bound calcium.

On the other hand, SAC Calcium can easily be broken down by the bond of atoms and can exist in the form of ionic calcium. It is easily dissolved in water, directly absorbed into the blood vessels through the gastric mucosa, and has the function of activating other protein-bound calcium through natural hormone action.

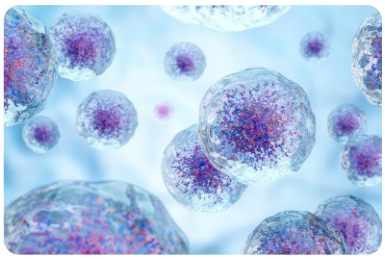
This ionized calcium activates osteoblasts and rapidly increases bone density. Over the age of 35, new bone formation does not occur, and this ionized calcium enables bone formation that has ceased.

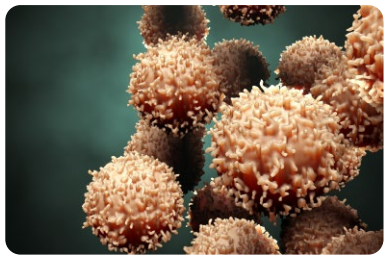
It can be seen that the absorption rate is 200 times higher than that of normal calcium. It has been shown that ionized calcium obtained through the intake of SAC Calcium activates osteoblasts and rapidly increases bone density.

Considering that ionic calcium deficiency causes osteoporosis and numerous diseases, the scope and effect of ionized calcium in our body is surprising.

I hope that this has helped to rectify common misconceptions about calcium that we may have had in the past, and I hope that it will offer hope to those who are currently suffering because of not having found a solution.

2. Stem Cells and Calcium





Stem cell therapy, such as growing human ears on the backs of mice and

growing organs in laboratories, has been introduced, and many people who do not suffer from incurable diseases have been given renewed hope, which includes the expectation of impending liberation from their disease. And now, more than ten years after the introduction of this treatment, what has changed? Why did a boy treated with stem cell therapy have to develop cancer?

In 2001, a nine-year-old Israeli boy with a rare disease, suffering from capillary dilatation dyskinesia, went to Russia to undergo stem cell clinical trials. Neural stem cells derived from the placenta of others were injected into his brain and spinal cord. However, the boy's health condition worsened and researchers at Tel Aviv University in Israel found cancer in the boy's brain and spinal cord. Researchers concluded in a paper published in the US Public Science Library's international medical journal PLOS Medicine that "allogeneic stem cells would have caused tumors in juvenile patients."

Many similar studies have shown that stem cells are alarming in terms of their medical safety. In 2009, a German team found a tumor in a pig injected with mesenchymal stem cells. In 2012, researchers from Belgium and Switzerland extracted myelogenous cells from mice, cultured them in the laboratory, and then administered them back into mice, resulting in conversion into cancer cells.

In Korea, there is a precedent in which two Korean patients died while RNL Bio was administering stem cell treatment in foreign countries to avoid the law. The company explained that patient death was not directly related to stem cell procedures, but the controversy did not abate.

Despite this, there are so many patients who expect stem cells to be beneficial to cancer. Countries around the world are continuing to study stem cells, investing heavily in avenues of stem cell research. One avenue of research is research into stem cells used for cartilage regeneration, which has few side effects due to the lack of blood vessels in cartilage. With the permission of respective authorities, application of stem cells in related procedures may result in successful recovery.

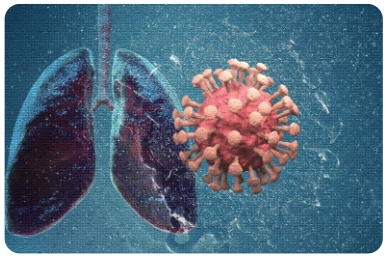
In the case of localized stem cell procedures, the survival of stem cells is closely related to the calcium levels of the tissue cells. However, even if many stem cells are cultivated and injected, if the calcium levels of the respective part of the human body is low, the injected stem cells can not

thrive in the corresponding site.

In the case of SAC calcium, some tissue stem cells were successfully applied in conjunction with SAC for a breakthrough in treatment, causing us to feel that the future of stem cell research is bright. The study of stem cells still has a long way to go, but the future of stem cells has become brighter with the emergence of SAC Calcium.

3. SAC and the Lungs





The British Prime Minister, Boris Johnson, during its time, delivered a message stating that coronavirus could separate relationships between loved ones. The aftermath of COVID-19 has illustrated many difficult challenges due to the nature of the uncertainties and unforeseen illnesses. During its peak, everyone including medical professionals and patients, were experiencing extreme fear due to this epidemic, which led to many deaths of individuals who had Covid-19. In the United States, confirmed cases have risen from thousands to millions of people, and many medical professionals have been infected. It was impossible to predict how much worse the situation could end up.

When an individual possesses the coronavirus, it first creates lung failure of the individual and as it gets worse without treatment, and ultimately leads to death.

Therefore, one must ask themselves, what role does calcium play in preventing this? We will investigate the role of calcium in the toxicity of the lungs.

The lungs accept oxygen from the air, which is moved into the bloodstream and exchanged for waste at the cellular level. The lungs then act to discharge carbon dioxide, which is waste in the blood, into the air.

Small blood vessels surround the alveoli that help exchange oxygen and carbon dioxide in the lungs. If the air flow in the bronchus is insufficient or the gas exchange is not performed properly in the alveoli, you will experience difficulty breathing, which in extreme cases could result in respiratory arrest and the loss of life. If toxic substances or viruses enter lung cells from outside the body, our body activates the immune function to fight them. Calcium homeostasis is very closely related to lung health.

According to a paper in a journal on toxicity-related research in 2015, when toxic substances enter the lungs at low calcium levels, the lung cells lose their function and the result is difficult breathing.

In other words, annexin V, which acts as an inflammatory inhibitor in normal lung cells, increases by more than 3-4 times over time due to the invasion of toxic substances, and is converted into negative proteins by binding with phospho-lipidase during the signaling process in the cell, which negatively affects lung cell metabolism, causing inflammation and making it difficult to suppress oxygen-carbon-dioxide-exchange in lung cells.

We will share information on the effects of SAC Calcium on lung disease patients, such as those with lung cancer and pulmonary fibroma, which has been obtained through clinical trials of numerous diseases during SAC Calcium studies.

An 84-year-old man with chronic pulmonary fibrosis was a man who lived in pain, relying on oxygen tanks. However, he was able to lead a normal life, without the need to rely on oxygen tanks, due to the comfortable breathing and sleep made possible through the supply of SAC Calcium. Additionally, an 80-year-old male patient who was suffering from terminal lung cancer was able to breathe comfortably due to SAC Calcium. A 60-year-old woman who had been suffering from lung disease for more than 30 years and was living on oxygen tanks just two to three years ago. She had been unable to lie down for the last two weeks, but [after using SAC Calcium] she said that the heavy feeling in her lungs seemed to disappear for a moment. Looking at these patients, we realize how important calcium is to our lung health. We are passing through a dark, long tunnel because of the coronavirus, but I hope that SAC Calcium will be a ray of hope.

4. Calcium, Pregnant Women and Fetal Health




A few years ago, when I attended a seminar in a Canadian city, I consulted a married woman in her mid-30s after the lecture. She had children that were six, four, and two years old, all of whom were autistic. It is difficult having just one autistic child at home but all three of her children were autistic.

According to statistics, the total population in Korea is decreasing, so why is the birth rate of children with disabilities increasing? Although the marriage age is increasing, the increase of children with disabilities is very worrisome. This is not only a problem for the families that experience such disabilities, but it is also a social and national problem.

Disability appears at birth or in growth. Diseases such as intellectual disability, brain lesions, vision impairments, pediatric diabetes, severe allergies, hearing loss, language impairments, mental disabilities, autism, kidney, heart, respiratory, and liver disorders, craniofacial disorders, enterorrhagia, and epilepsy are of increasing concern. How could this happen? Life begins with DNA replication and cell division. DNA replication constantly occurs, but if DNA is not replicated normally, mutations can increase the likelihood of disability. The number of cells in newborns is about two trillion. Human genes consist of 19,950 protein coding standards. So how much normal replication will take place when many of these genes are replicated? And how much abnormal replication will take place? Calcium helps to deliver normal information when DNA is replicated. When calcium is lacking, normal replication does not occur.

During pregnancy, calcium requirements are doubled: if the usual calcium requirement is 800 mg, an excess of 1,600 mg is needed during pregnancy. Assuming that pregnant women absorb 100% of the calcium,

if the newborn weight is 4 kgs, the bone weight is about 800 g, of which pure calcium is about 80 g. The amount of calcium consumed by the pregnant woman for 270 days is 432 g, of which, about 216 g of calcium is consumed by the pregnant woman. Aside from 80 g of calcium used in bone formation, 136 g is the amount of calcium used from the time of conception to birth. In other words, the fetus consumes an average of 503 mg of calcium per day for cell division and metabolism. If you supply a smaller amount, you may have problems with DNA replication, cell division, and metabolism in the fetus. When calcium insufficiencies exist for extended periods during pregnancy, children may experience disabilities at childbirth or during growth. Calcium supplementation, which plays a role properly before and during pregnancy, is indispensable for healthy child birth.

5. Cancer Therapy Patients and Heart Function





One summer in mid-July, an attractive, petite woman in her mid-thirties visited the lab along with her parents. This woman, who was also the mother of two children, had been diagnosed with late-stage gastric cancer in May. She had received several chemotherapy sessions and had come to see the doctor, who had told her that she could no longer be treated. Her face was pallid and looked as if she were going to collapse at any moment. The parents, who heard the young woman say “I want to live,” would have likely felt like collapsing as well.

I mustered up the courage and asked them to try and take SAC Calcium starting from that day. One day, a month later, as I was opening the door of the institute, she appeared and said, “I went to a barbecue picnic and my friends thought I was a ghost, I was so surprised. Some friends were

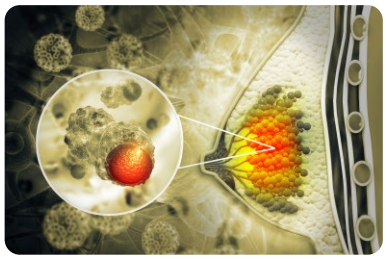
too afraid to call." I can still see how happy she was that her health had somewhat recovered. But, one day in October, she died after 24 hours of injections in the hospital.

Many anticancer drugs are toxic to the heart. The heart is extremely sensitive to the drugs or injections we take. The electrical signals of the heart or the calcium signals that move the heart muscle are immediately affected when harmful substances enter the blood. So doctors should check the ECG (eCG) and heart functions of patients before prescribing such drugs, as they may lead to death. In recent years, high-dose vitamin C has been injected as an alternative treatment. In addition, patients with G6PD deficiency may face a situation in which red blood cells break down (hemolysis) or occasionally cause heart attacks. The number of people who are G6PD-deficient worldwide is estimated to be 33 million, and patients who are congenitally lacking G6PD (glucose-6-phosphate dehydrogenase) should be aware of this factor when injecting vitamin C intravenously.

They don't know what killed the young woman, and the hospital said they didn't know why she had a heart attack, as cancer cells hadn't grown and her heart was normal. The heart is the most important organ in our body. When we administer toxic drugs, we must recognize their toxicity in advance.

6. Calcium and Breast Cancer





The population of people being diagnosed with breast cancer is more than four times higher than 15 years ago. Women are not free from the threat of breast cancer as they get older, due to stress, environmental pollution, and the overwhelming abundance of processed foods. These days, young

women are unknowingly affected, surprising those around them.

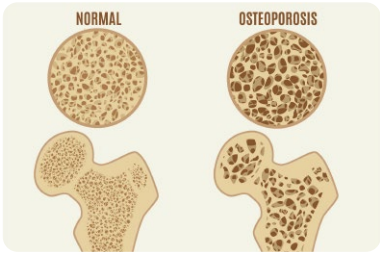
Calcium regulates the body's hormone levels through normal muscle, DNA replication, and signaling. Among the hormones, female hormones are closely related to breast cancer. Drugs using female hormones have been developed and prescribed for osteoporosis treatment, but they are now rarely prescribed due to concerns over breast cancer. This is also evidence that a hormonal imbalance can cause breast cancer.

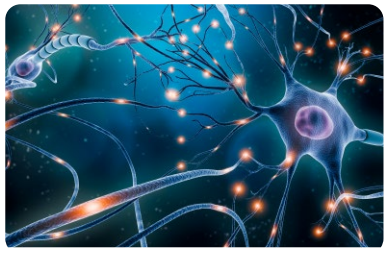
Female hormones have three main endogenous estrogens that have hormonal activity, these are: estrone (E1), estradiol (E2), and estriol (E3). Among these, estradiol (E2) is the most common form in non-pregnant women; estradiol levels vary with menstrual cycles and are highest just before ovulation.

The primary estrogen from menstruation to menopause is 17-estradiol. When a woman experiences menopause after passing her reproductive age, estrone (E1) becomes a major estrogen. Estrone is weaker than estradiol. Estriol is the main estrogen during pregnancy. Estriol is considered to be a weak estrogen. Estrone is known to act as "bad" estrogen and may be the cause of this estrogen-related cancer.

In my study, I observed that many breast cancer patients recovered through use of SAC Calcium by reducing estradiol in osteoporosis and increasing estradiol similarly to experiments done with mice. An increase in estrone along with a decrease in estriol causes cancer, which can be seen as a proven hypothesis that the increase in blood estradiol can calm cancer. As a result, estradiol is a precursor to estriol by ionic calcium, and estriol is a "good" estrogen, which is an important hormone for preventing and restoring cancer. Maintenance of estrogen is closely related to calcium levels. It is redundant to tell women how important intracellular calcium is to health.

7. Osteoporosis and Memory





Most women begin to recognize a decline in memory as they experience menopause, but they usually accept it as a natural phenomenon that happens as they get older. But dementia, Alzheimer's in particular, is another problem.

There are research papers that suggest memory and cognitive decline is closely related to bone health. Recently, a study has been published that links osteoporosis to memory. Professor Gerard Cassinti, of the University of Columbia (USA), has published a study about how a lack of osteocalcin in the bones leads to memory loss. This is a phenomenon that occurs as bone density decreases. Professor Cassinti showed that in the clinical trials of mice without osteocalcin and mice with osteocalcin, the memory of mice without osteocalcin scored very low in cognitive and memory tests.

Osteocalcin is known to promote insulin secretion and increase insulin sensitivity, which is known to play a role in promoting glucose metabolism.

This is the first study where osteocalcin is shown to be involved in memory. This also gives hope for a broader perspective of preventing the decline in memory loss and cognitive ability as one gets older. If we believe in Professor Cassinty's mouse experiments, osteocalcin seems to be able to improve memory and provides hope for patients diagnosed with Alzheimer's disease.

CBHI has received numerous feedback in regards to the improvement of memorization and concentration within children, students, and the elderly who take SAC Calcium. In clinical trials of experimental mice that we have studied, we can see that osteocalcin increases, which supports

the above feedback. As such, bone health is not a problem of fractures, but these various substances, from hormones to calcium supply, such as osteocalcin, osteoblasts, and osteoclasts produced in the bones, which play an important role in our body's overall metabolism, such as DNA replication and cell metabolism.

Bone health! It is an important factor for those that are interested in living a healthy life as you get older.

8. You want to live long? Calcium and Life





Even if we do not mention the "elixir of eternal youth", it is the ultimate hope for any and all people to live a long and healthy life. Last week, I saw a disabled woman with a deformation of her arm and hand at the Frankfurt Airport in Germany, and I thought that she may have been lacking in calcium supplies while she was still a fetus. The proper development of the body is important, and calcium supply in the cell is an essential factor in cell metabolism. If cell metabolism function is reduced due to a lack of calcium, the cell degenerates or causes inflammation through the operation of the autoimmune system, which is the beginning of degenerative diseases.

So the Creator stored calcium in the bones when he made our human body, and designed it in such a way as to release parathyroid hormones whenever necessary to remove calcium and use it in the necessary parts.

A few years ago, a Danish research institute conducted a research study on the average lifespan of 35,000 people, divided into healthy people and unhealthy men and women. According to the study report, people with healthy bones in their 50s have an average life expectancy of 11 years and 6 months longer than those who are not healthy. This suggests that

calcium supply in the human body is an absolute factor in bone health.

The bone density of degenerative disease patients and cancer patients is lower than that of normal people, resulting in complex diseases as well as one or two other concomitant diseases. Bone density is not only a factor leading to bone fractures, but an insufficient calcium supply also affects brain cells and the metabolism of all cells, which causes various diseases. Calcium = Life, and this is not going to change as long as mankind is alive on this earth.

9. Food and Calcium





Human beings eat and live on earth, and as crop production has become commercialized, humans have been turned to pesticides to produce more abundant crops. In addition, we have used genetically engineered crops to save costs. While we hope to revert to organic crop production, it is difficult to realize. All plants have a defense system to protect themselves from external bacteria and pests, which is the result of plant defense hormones. Now, well-meaning farmers are aiming for organic cultivation, and that small output (3-5% of the total agricultural market) is in the market.

During China's 19th National Congress in 2018, Xi Jinping announced that 60% of China's health care expenditure was on preventive medicine. It is a recognition that Chinese medicine is struggling in the competition between Chinese medicine and Western medicine. It also means that Chinese herbal medicines grown using pesticides and fertilizers have become difficult to utilize as medicinal materials. Only 1 ounce of a medicine used half a century ago would not have the same effect today, even if you use 10 times the previous amount.

What about agricultural products? The nutrients from all grains, vegetables, and fruits, including potatoes, tomatoes, corn and apples, are reduced to 1/10-1/50 compared to that 50 years ago. But more serious than that is the problem of pesticide residue in vegetables and fruits, which has caused many diseases including cancer. Recently, 15 brands of beer were tested for pesticides, and most beer and wine brands were found to contain the ingredients of Monsanto's herbicide Roundup (glyphosate). Brands with residual pesticides detected include Budweiser, Coors, Miller Lite, Sam Adams, Corona, Heineken, Guinness, Stella Artois, New Belgium, and Samuel Smith Organic. Also included were Beringer, Barefoot, and Sutter Home. Since the use of disinfectant herbicides is essential for raw materials used in beer, such as hops, and grape farming, pesticides are detected in the final product. However, pesticide companies and liquor producers are protesting that they meet the pesticide residue standards. Monsanto has marketed Roundup as harmless to the human body, and in August last year, a school gardener who had been diagnosed with cancer was awarded a settlement of $289 million by Monsanto. Monsanto has also faced more than 5,000 subsequent lawsuits related to this. The residue of pesticides in food requires more calcium consumption in human metabolism, and diseases caused by calcium deficiency in the human body have increased from cancer to osteoporosis, cardiovascular disease, dementia, and stroke, which threatens young people, the elderly, and even children with rare diseases. Therefore, not only vitamins and minerals, but also the right calcium supply to the human body is essential in this day and age.

10. Osteoporosis Treatment Prolia (denosumab)





According to the International Osteoporosis Foundation, approximately 200 million people worldwide are estimated to have osteoporosis, with osteoporosis-related fractures occurring once every three seconds, amounting to more than 8.9 million cases annually. Thirty percent of women and 20% of men in their 50s or older experience osteoporotic fractures at least once. As osteoporosis is a prevalent condition among the elderly, it is expected to become a significant public health concern in future societies due to the rapidly aging population. Its prominence is growing alongside the increasing prevalence of Sarcopenia.

The osteoporosis treatment market in the seven major markets, including the United States, Japan, and Europe, witnessed an increase from $6.15 billion (approximately KRW 6.7 trillion) in 2014. It is expected to reach $9.34 billion (KRW 10.2 trillion) by 2024. Osteoporosis has a lower diagnosis and treatment rate compared to other diseases, but it is expanding due to the growth of the elderly population, increased awareness of osteoporosis, and rising healthcare expenditures.

In recent times, it is likely that very few individuals suffering from severe osteoporosis are unaware of Prolia.

Fourteen years after the launch of Prolia (denosumab), an osteoporosis treatment drug from the pharmaceutical company Amgen (AMGEN), which received approval in June 2009, Prolia is now listed on NASDAQ as of January 31, 2023, under the ticker symbol AMGN. According to the financial report, sales amounted to approximately 1.4 trillion won (2022), with 110 billion won sold in Korea alone in 2022.

Prolia is a medication commonly prescribed by many doctors as an osteoporosis treatment due to its more potent osteoclast inhibition

compared to bisphosphonates. Detailed clinical research results for Prolia can be found in the journal "J. Clin Endocrinol Metab," specifically in the April 2011 issue, Volume 96, Issue 4, pages 972–980.

This research paper involved a total of 256 postmenopausal women representing various ethnic backgrounds, including white, black, Latino, and Asian. The study spanned a 48-month period and took place at universities and research centers in Canada and the United States, with the participation of Amgen. The clinical trial's conclusion revealed an increase in bone density of 3.5-6% over a 24-month period. However, it was observed that once the drug was discontinued, bone density rapidly decreased. In other words, the conclusion of this clinical study is that if you are prescribed and administered Prolia but discontinue its use due to side effects, it is imperative to promptly obtain a prescription for another alternative bone resorption inhibitor or bone-forming agent. As for the increase in bone density following Prolia injection, in the aforementioned clinical trial, the overall average T-score for lumbar spine BMD (bone mineral density) improved from -1.66 to -1.56. However, the author refrains from definitively characterizing this change as either an increase or merely the maintenance of bone density. I feel profound concern when I observe health authorities seemingly supporting pharmaceutical companies that generate billions of dollars in profits through these questionable treatments, and when I see patients passively receiving prescriptions and bearing the burden of medical expenses. I will leave the judgment of these matters to the readers.

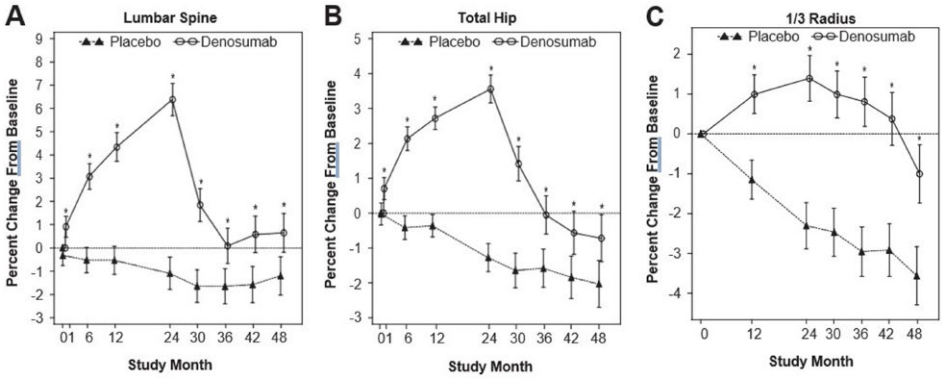


Figure 157. Percentage change from month 0 in Lumbar Spine (A), Total Hip (B), and 1/3 Radius (C) BMD over 48 months. Percentage change from month 0 in BMD is presented as least squares means and 95% confidence intervals based on a repeated measures model that adjusts for treatment, strata, visit, month O value, densitometer type, treatment-by-visit interaction, and month 0 value-by-densitometer type interaction. Includes participants enrolled in the off-treatment phase with observed values at month 0 and the time point of interest [n = 110-128 (placebo) and n = 109-128 (denosumab)]. *, P < 0.0071.

According to the study published in J. Clin Endocrinol Metab, April 2011, 96(4):972–980, the graph illustrates a 3-6% increase in bone mineral density in the lumbar spine and thigh, along with a rapid decrease in bone density upon discontinuation of the treatment.

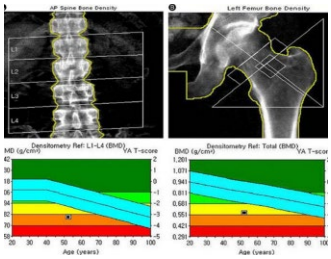
What remains to be considered are the effects of these treatments on bone quality and the potential side effects of the medication.

Normal bone health relies on the continual replacement of old bone cells with new ones. These medications typically deactivate osteoclasts within the bone, allowing old bone to persist and gradually leading to a decline in bone quality, which can ultimately result in atypical fractures. In cases where fractures occur due to a fall, the deteriorating bone quality can cause the bone to become brittle or powdery. Furthermore, the side effects of these medications can vary from person to person, and many individuals have expressed profound distress at the severe side effects they've experienced after being prescribed these medications. (For more information, please refer to reviews on www.drugs.com.)

We sincerely hope that when your doctor prescribes osteoporosis treatment medication, you will be able to make an informed decision for the health of yourself and your family by seeking and considering a variety of information.

11. DEXA (Dual-energy X-ray absorptiometry)





An adult weighing 70 kg loses and regenerates 5-6 g of bone a day. However, as we age, if more bone is lost than filled, the bones become porous and can eventually break easily.

As we live in an era where life expectancy has increased to 100 years, it seems like 30 to 40 years have passed since we faced the challenge of osteoporosis, a degenerative disease that typically occurs in people in their 50s or 60s. Numerous research institutes and pharmaceutical companies around the world are working diligently to address this issue, conducting clinical trials, and releasing numerous treatments, but there is still no treatment with a definitive solution.

Osteoporosis is a degenerative skeletal disease characterized by low bone mass and microstructural changes that render bones brittle and increase the risk of fractures. Today, due to scientific advances and an aging population, the number of people suffering from chronic bone diseases has significantly increased, placing a substantial burden on the healthcare system. This condition has cost the Canadian government more than $8 billion (as of 2015), while in the United States, the cost is $30 billion, and in Korea, it amounts to $1.2 trillion (as of 2012).

Therefore, for chronic diseases, especially osteoporosis, it is of utmost importance to focus on prevention and early diagnosis to mitigate complications and the resulting social health costs. Consequently, there has been a recent surge in interest in identifying new parameters and techniques capable of diagnosing osteoporosis and predicting the risk of fragility fractures in a simple and economically sustainable manner.

We will explore the reliability of DEXA (Dual-energy X-ray absorptiometry), an imaging technology widely employed to assess bone health.

She was scanned using an ultrasound machine owned by CBHI and

found that her bone density was completely normal. She says she has osteoporosis and I wonder what would have happened if she had purchased and taken her prescription medication. She was not at a small hospital, but at a large hospital, the DXA machine diagnosed and prescribed a prescription with incorrect measurement values. I think she must have been perplexed. Of course, there is a good chance that the hospital where the measurements were normal will also obtain different numbers the next time they are taken.

DXA dual-energy X-ray absorptiometry measures bone mineral density (BMD) and is universally recognized in hospitals around the world as the gold standard for the diagnosis of osteoporosis. But unfortunately, DEXA has too many error conditions.

Eur Radiol 2015 May;25(5):1504-11. doi: 10.1007/s00330-014-3509-y. Epub 2014 Nov 27.

Prevalence and types of errors in dual-energy x-ray absorptiometry.

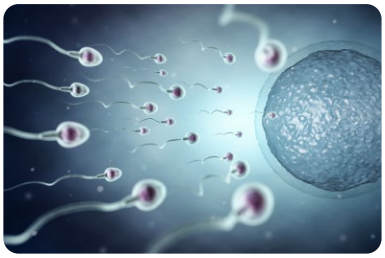
The conclusion of the 2015 study was that over 90% of the 2,476 patients measured with DEXA had errors in their measurements. Additionally, the study found that 90% of DXA exams experienced errors, including issues related to establishing patient geolocation information, patient positioning, and analysis. Additionally, certain factors, such as patient motion during the scan or variations in equipment calibration, could lead to significant errors in DXA results.

For instance, aortic calcification can result in an overestimation of bone mineral density, particularly in cases of vertebroplasty involving more than two lumbar vertebrae, impacting the reliability of accurately assessing BMD using DEXA, especially in elderly patients. One common cause of complex numbers when using DEXA is osteoarthritis (OA).

In bone-related clinical trials, I have experienced many cases where blood bone metabolism indicators and DEXA measurements did not match. Among them, the biggest error was that the measuring instrument was not calibrated properly before DEXA measurement, or the frequent replacement of the measuring instrument was the main error.

Living in the age of 100, we have to worry about our bone health for more than half of our lives. How can we trust the current medical technology and system and entrust our bone health to it? This is a point of great concern.

12. Calcium and Infertility





One day, a woman over 70 years of age called and expressed her thanks. She communicated that her daughter, who was over 35, had a baby after five years of marriage. It seems that they had been taking calcium for more than four months, and even though infertility in couples may vary from situation to situation, some couples have children simply as a result of the proper calcium supply.

Developed countries, except for countries that accept high rates of immigration, are worried about population decline. In addition, even if marriage is avoided due to economic reasons, there are emerging concerns from many young people and newlyweds who worry about infertility.

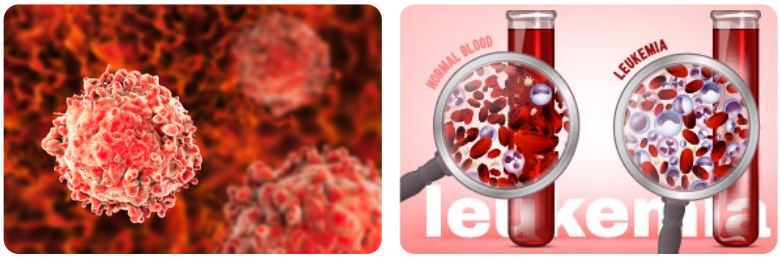
Why is the number of infertile couples increasing? There may be many causes of infertility, but in this column, I would like to talk about calcium supply in the human body. Starting from about 20 years ago, obstetricians have studied fertility and have given attention to the number of sperm in the case of young married people, and have studied the quality of women's eggs. The number of sperm has decreased significantly compared to 50 years ago, and the rate of egg production has also increased. In the case of male sperm, the amount of calcium in the semen surrounding the sperm has decreased from 150 million to 100 million, and even to 50 million in one case.

When fertilized, cells within the egg begin meiosis by creating calcium signaling (Ca^{2+}wave) with a significant increase in Ca^{+} ion concentration. If calcium is not properly supplied, growth of fertilized eggs cannot be expected: calcium deficiency can cause infertility. In addition, Ca^{2+} increase in eggs is increased by Ca^{+} in the endoplasmic reticulum by interfering with IP3 receptors, and Ca^{2+}, which is a leading factor in the

induction of Ca^{+} increases, is likely to be transferred to eggs by sperm cell proteins. Uncorrected mature eggs are stationary for a certain period of meiosis and do not move in a metabolically immobilized state. They are freed from the stationary state by calcium signaling and are divided through meiosis. The concentration of Ca^{2+} in oocytes dramatically increases with the initiation of fusion, and the calcium signaling plays an important role in DNA information transmission during DNA replication by transmitting signals in oocytes. So, in fertilization, Ca^{+} ion by calcium supply is a clear indicator that infertility is not signaling or insufficient because of the low calcium ion level.

As a result, the cause of infertility is that calcium deficiency is absolute and dormancy does not immediately fuse sperm and eggs. Even if sperm and eggs are fertilized, meiosis does not occur if the calcium signal does not reach the fertilized egg cells. In addition, if the calcium concentration is low in the semen surrounding the sperm, the activity of the sperm becomes very low and can not cause calcium signals. This is the key mechanism of fertilization and the point of embryo growth: if the fetus grows normally during pregnancy and then suddenly faces a problem, it is necessary to check the thyroid function of pregnant women. This is because there is a problem with calcium supply.

13. Leukemia and Calcium (I)



Ten years ago, I received an international call from a Korean missionary. It had been 72 days since the grandson of this missionary was born, and the baby was diagnosed with leukemia at a large general hospital in Gangnam. He said the baby had received all available treatments, but he had no hope. The missionary said he wanted to feed [SAC Calcium to]

the sick baby. I asked him to use a bottle to feed it. A week later, the baby's grandmother called, and said the baby's white blood cell count had risen to 300, to 600, to 900, and then to 1,200, and she was delighted. A month later, the white blood cell number normalized and the baby was discharged from hospital. The boy is now well and in primary school.

Another case is a 71-year-old man who is a dentist in Vancouver and who was being treated for chronic leukemia. He confined himself to his house because he could not dream of going out. After taking SAC Calcium, his white blood cell count rose and after a month, he went for a walk of about 4 km round trip. Now, five years later, he still attends church, travels long distances, and is enjoying a healthy old age.

Leukemia is a type of hematologic tumor in which leukocytes multiply abnormally in the blood. In general, non-solid cancers that occur as high numbers of unmatured leukocytes in the blood are called leukemia. Therefore, the average white blood cell count is very low. The normal blood cell count is extremely small, so much so that the existing cells can not perform basic blood functions such as oxygen or nutrient transport as well as immune functions.

In addition, abnormal white blood cells cause reactions similar to autoimmune diseases, which destroy normal tissues. When chemicals and radioactive materials are nearby, the possibility of developing leukemia is increased because of the decrease in the intracellular ionic calcium levels due to these carcinogens, resulting in abnormal proliferation in hematopoietic stem cells-multipotential stem cells-myeloid cells, and the lymphoid stem cell differentiation process in the bone. Leukemia is largely divided into four categories.

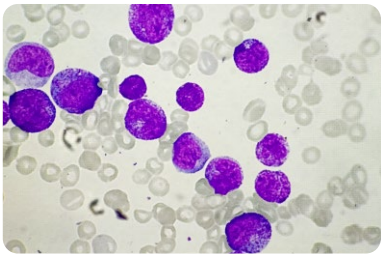
- Acute myelogenous leukemia (AML)
- Chronic myelogenous leukemia (CML)
- Acute lymphocytic leukemia (ALL)
- Chronic lymphocytic leukemia (CLL)

In myeloid stem cells, red blood cells, platelets, white blood cells, etc. are differentiated from each other, and differentiated from lymphoid stem cells to B cells, T cells, and NK cells. Calcium is an indispensable material when differentiated into cells.

So what is the cause of leukemia? It is known that there are some

genetic, chemical, and radiological causes, but there is no obvious cause. However, I am confident that when calcium is an issue with the calcium allele of mycelium, it acts as a trigger for leukemia. Based on the incidence of leukemia in children and leukemia after the age of 50, problems with calcium alleles, such as stem cell differentiation, calcium signaling in DNA replication, and information transmitter roles, are emerging. In other words, a lack of calcium ingestion during pregnancy, or a lack of calcium in those who are in their 50s or older, can be said to lead to an abnormal proliferation of white blood cells.

14. Leukemia and Calcium (II)

Blood cancers, such as thrombocytopenia and aplastic anemia, also begin with bone marrow. In most hospitals, chronic leukemia usually influences the dosage of Gleevec® administered to control the patient's situation, but it is not a cure-all. The patient uses lifelong-dosing to control symptoms. If Gleevec® is not effective at all, hematopoietic stem cell transplantation (HSCT or HPSCT) is a last-resort therapy to kill all white blood cells in the bone marrow and to transplant bone marrow. The transplant itself is dangerous. Transplantation can fail, and it can be said that there is always room for recurrence, or sepsis, even if the transplantation is successful. There are limits with current medicine, but the average 5-year survival rate has been increased to about 90% through the introduction of Gleevec®.

In particular, acute leukemia has a poor prognosis for all treatments, including Gleevec®, and the average survival rate is usually less than seven months. The cause of leukemia begins with bone, and is also known to start with bone marrow hematopoietic stem cells.

Therefore, the cause of leukemia is calcium. I am sure that a treatment that does not include calcium will not be optimal. The benefit of introducing SAC Calcium is increasingly solidified through several clinical trials, from bone marrow transplantation to patients using Gleevec®. Calcium in bone and bone marrow is so vital that it can not be truly expressed in words.

15. Calcium and Eye Disease





I do not know of other organs in our body that age as rapidly as the eyes. After the age of 40, the eyes slowly degenerate. Some people must wear reading glasses to see printed text, and some people with short-sighted vision must remove their glasses to do so. I remember Helen Keller talking about it. With the exception of when we are asleep, we generally keep our eyes open and freely observe nature, the faces of family and friends, and the things around us. We take for granted how precious it is to be able to see.

I just had a conversation with an ophthalmologist.

The ophthalmologist said that he had done more than 2,500 ophthalmology operations, and he was proud of being an expert on eye surgery, but he felt a sense of discomfort while treating eye disease patients. The reason is that eye disease does not improve, but simply stops worsening or deteriorates more slowly. There is nothing more that can be done after treating patients, so I feel a sense of discomfort as a doctor.

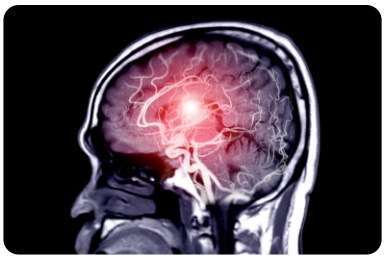
As you get older, you often visit the hospital for degenerative diseases, and eye diseases are no exception. The most common ophthalmologic diseases include cataracts, glaucoma, macular degeneration conjunctivitis, uveitis, and retinal pigmentation. Also of note are denaturation, ocular

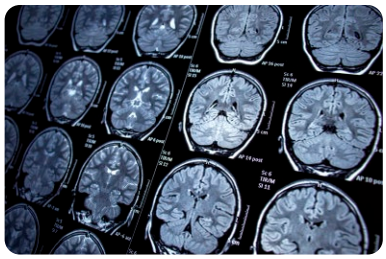
pressure, dryness, retinopathy, keratoconus, ophthalmitis, etc.

According to reports by the US Eye Research Institute, which says that degenerative eye disease is very closely related to calcium alleles in the human body, the supply of calcium as people age is a very important issue. When calcium is supplied to children with poor vision, their vision improves.

In terms of normal recovery, the effects of calcium on optic nerves, the retina, and inflammatory diseases are undisputed, even if one does not read calcium and ophthalmologic papers. Calcium! It is time for the right choice.

16. Calcium and the Brain





Most people grew up listening to their parents nagging them to study hard when they were young. We have heard from the news that even a good student who did not need to be nagged has committed suicide due to the pressure of studying. But how can you learn well when your brain is not able to properly receive the information you are learning?

As I mentioned, calcium is a neurotransmitter that is indispensable to brain cognition, memory, and concentration, and a lack of calcium degrades these abilities. What is concentration? Depending on the activity status of the brain, brain waves are divided into alpha waves, beta waves, theta waves, and gamma waves. When in the state of the theta waves, concentration is at its optimal state. What is the relationship between ionic calcium and alpha waves? Many studies show that increasing the amount of ionic calcium supplied to the brain increases the amount of alpha waves emitted and increases concentration and acuity. Alpha wave: Concentration, memory, cognition, and acuity. What kind of brain

waves? Depending on the state of activity of the brain, the brain waves are alpha waves (7-14 Hz), beta waves (14-30 Hz), delta waves (0.5-4 Hz), and theta waves (4-8 Hz).

If the level of arousal is too high, as when you are excited or nervous, the brain waves produce what is called beta waves and delta waves are usually seen when an individual is sleeping. The brain waves that appear where the level of arousal is not too high or too low are alpha waves or theta waves. Therefore, the level of arousal that can properly maximize the efficiency of information processing, memory, and concentration of the brain is the brain state in which relatively low brain waves, such as alpha waves and theta waves, are emitted.

In order to improve the performance of learning that requires comprehensive thinking, good textbooks are important, but above all, the brain conditions, which are the basis of learning, should be suitable for learning. As in sleeping, the level of arousal of the brain is low, or the level of extreme arousal, such as excitement and tension, is rather low. This is due to a lack of calcium and can be solved by supplementing SAC Calcium.

17. Hypercalcemia, Urology and SAC Calcium





As I have already mentioned in these columns several times, blood calcium levels are very important to maintaining metabolism in the human body. This blood calcium concentration is largely regulated by parathyroid hormones, vitamin D, and calcitonin through thyroid hormones. Calcium in our body is absorbed through the small intestine and excreted through the kidneys, and the bones act as a storage facility for storing calcium.

In addition, bone health is metabolized and maintained through this.

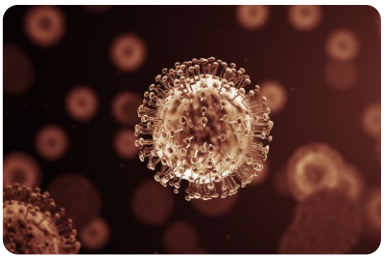
Therefore, abnormalities in hormones and organs that perform this role cause changes in calcium concentration. Among the various causes, malignant tumors such as lung cancer, breast cancer, myeloma, leukemia and hyperparathyroidism account for more than 90% of the total. Other diseases such as chronic renal failure and hyperthyroidism, terminal hypertrophy, brown cell tumors, adrenal dysfunction, or excesses in drugs such as vitamin D and A, thiazide diuretics, lithium, and breast cancer hormone therapy can also occur.

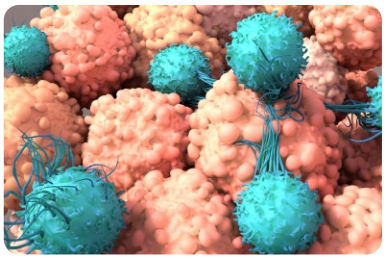
Clinical symptoms of hypercalcemia are non-specific and similar to symptoms of several diseases. Normal human blood calcium levels are 8.5-9.5 mg/dL, and mild hypercalcemia (11-12.0 mg/dL or less) does not usually cause the manifestation of symptoms, but sensitive people often complain of nausea, dehydration, polyuria, constipation and anorexia. If the concentration of serum calcium increases to 11-12.0 mg/dL, the following symptoms include general weakness, fatigue, depression, impaired consciousness, hypertension, arrhythmia, pancreatitis or ulcers. And if the concentration increases to 13-15 mg/dL or more, the patient may lose consciousness.

In hospitals, treatment of hypercalcemia begins when the blood calcium concentration is 13-15 mg/dL or more, and by supplying proper moisture using a saline solution, calcium is discharged into the body through the kidney. This treatment may be accompanied by hypokalemia and serum hypomagnesemia, so it should be periodically checked during treatment: aiding calcium release, and overhydration in the body due to water supply.

Furosemide-type diuretics are also used in prevention. If treatment is difficult due to the deterioration of heart and kidney functions, it is treated with hemodialysis, and medication is administered to inhibit the function of osteoclasts that increase absorption in the bone.

18. Calcium and Viruses





It seems as though viruses existed before sentient life on earth began. We [have never been free from viruses and have been living with virus-related diseases since we were born.] The common cold is the most prevalent virus-related disease.

Why do we catch colds caused by viruses?

When our body temperature drops below a certain degree, we may notice that we get cold, cough and feel chills.

At that time, our body uses mitochondria to create a chemical reaction that raises our body temperature. Calcium ions needed for this reaction are quickly transferred from the bones into the blood and supplied to the mitochondria. There is another substance that is concurrently involved in this process. This is the substance called phosphorus, which is what the virus feeds on. The virus proliferates rapidly because of the high density of phosphorus in blood, and this is the reason why we catch the cold.

A student who was in his second year of high school had been suffering from the disease aplastic anemia. The only way to treat this disease was a bone marrow transplantation. However, after taking SAC Calcium, he recovered from this disease, and after graduation from university he was hired by a Gangnam-area general hospital in Seoul, South Korea.

The MERS crisis occurred in 2015, and during the crisis, the young man was exposed to the first case of the MERS virus at the hospital. Interestingly, this young man was not infected with MERS, whereas other doctors and nurses around him were infected and went into isolation. Hospital officials questioned how a person directly exposed to blood was not infected with MERS, while the doctors and nurses around him were infected. The young man was still taking SAC calcium.

Viruses start with RNA, a gene structure. RNA is made by combining ribose and nucleic acid with phosphoric acid, which is formed from a complete form of particles on the outer wall of the cell, along with sodium. This is called a virion or virus particle. The proliferation of viruses proliferates in the form of copolymerization, in which ribose and nucleic acid are combined around phosphoric acid. Viruses cannot generate energy or synthesize the proteins required for metabolism, so they proliferate by creating protective membranes using proteins that already exist. In the case of proliferation, viruses must be subject to absolute conditions in the body fluid environment to proliferate through extracellular fluids. The environmental conditions of proliferation are abundant phosphoric acid and a low temperature (below 36 degrees Celsius).

Not creating this condition is to prevent the virus from proliferating using calcium ions.Calcium ions play an absolute role in the reaction of sugar + oxygen in mitochondria, helping to increase body temperature and inactivating active RNA. In Europe, in the 18th century, cold patients were injected with calcium. At that time, calcium was combined with proteins in the plasma, and although its use was discontinued because of a condensation phenomenon, it seems that calcium has been useful for combating viruses for a long time.

SAC Calcium, which inactivates the virus, also inactivates RNA, because it increases plasma ionized calcium levels and increases the calcium levels of intracellular fluids to bind to phosphoric acid.

5 mg of SAC Calcium supplies 1.25 x 1019 calcium ions to and from the blood and cells at a time; in cases of serious infections, it may require continuous supply every three hours.

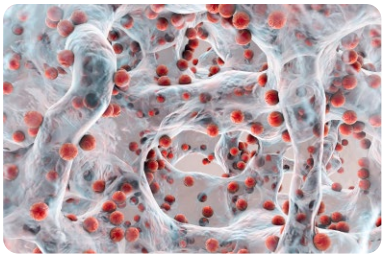
HIV viruses are no exception: HIV viruses are extinguished when there is a consistently high volume of plasma ionized calcium present. Of course, it is not possible to conclude whether the incubation period is prolonged or inactivated, but we have performed several clinical trials for the HIV virus so as to confirm that there is a significant effect.

“We could see a change in one patient after taking SAC Calcium for six days. His speech became clearer and he was able to do simple household tasks and go for long walks.”

The patient was seen by me during my visit to Tanzania in October 2019, and his nurse reported that he is still living a healthy life. Based on

these significant effects, it is expected that SAC Calcium would be a viable option for use in detailed clinical trials for HIV and AIDS management. Nowadays, a novel coronavirus in Wuhan, China, has spread not only within China but also throughout the world, raising concerns about its seriousness. Moreover, Asian discrimination is a current issue. If the epidemic continues and there is no vaccine or treatment, there may be serious consequences not only throughout China but also in many countries. I hope that SAC Calcium, which may provide good virus prevention and treatment effects in the future, will be made known to the entire world through formal viral clinical trials and that people will no longer have to suffer from viruses.

19. SAC Calcium and Microbial Biofilms




Why does resistance to various drugs, antibiotics, and antiviral drugs occur?

We have seen the spread of the Wuhan coronavirus, 2019-nCoV, in Korea and other countries, and COVID-19 infections terrorizing the world at an alarming rate.

Yesterday, TV news showed that the virus caused a patient's lungs to rapidly turn color, almost entirely white, in a single day. In the early stages, a virus invades a human body, and then when the body becomes a suitable environment, the viral population increases rapidly as it replicates.

Viral populations form a cluster and create biofilms on their own, and the biofilms have the effect of blocking the action of immune cells, antiviral agents, antibiotics, etc. So [as] this virus cluster tries to protect itself, it [renders] antiviral drugs and antibiotics useless. Of course,

some drugs can destroy biofilms and kill viruses, bacteria, etc. This is only possible if the drugs enter our systems in the early stages of a virus' infiltration of our bodies, killing it before it creates a biofilm. Was it because of a lack of antiviral drugs, antibiotics, etc. that many doctors in Wuhan died from the virus? It is estimated that viruses have already formed biofilms to block antiviral drugs, leading to death as the number of viral populations increases sharply.

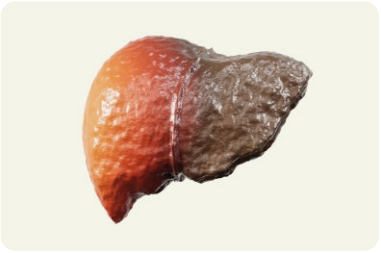
To date, many patients who have been diagnosed with COVID-19 in Korea and China have recovered and [have been] discharged because the medical staff responded during the initial stages of the infection and the antiviral agent was effective while the viral population was low and before the biofilm formation. However, the rapid increase in the number of deaths in China is attributed to the fact that the initial response to the patient's symptoms was wrong due to the lack of medical facilities. Many vaccine labs are conducting extensive research into a biofilm-destroying substance. Unfortunately, such a material has not been developed yet.

A 10-year-old boy with Lyme disease came to the research center with his mother two years ago. The boy had been taking various treatments over a period of two years from when he was diagnosed with Lyme disease, but he [had] not seen any effects from the treatments. Lyme disease is a viral disease that [can be unresponsive to antibiotics]. This is because bacterium Borrelia burgdorferi form biofilms and antibiotics do not work.

Three months after the boy took SAC Calcium, he had experienced nearly a complete recovery. Therefore, the basic clinical results obtained were that calcium ions can destroy biofilm. On January 29, 2020, at the Yanbian Hwaryong, Domun Hospital in China, two young men, aged 29 and 27 were being diagnosed with the 19-nCoV virus and were being treated in isolation at the hospital. We were given permission by the hospital and the Bureau of Osteopathic Hygiene to treat the two men with SAC Calcium. [They were discharged a few weeks later on February 9, 2020. This thus provides further confirmation.] I am hoping that the SAC Calcium will enable them to live free from fear of the virus. Many vaccine labs are conducting extensive research into a biofilm-destroying substance. Unfortunately, such a material has not been developed yet.

20. The Liver and Calcium





Two serum proteins measured to assess liver function are albumin and globulin; wherein, the total is albumin; the rest is globulin; albumin is produced only in the liver and is the most abundant plasma protein circulating in the bloodstream. Albumin is essential for maintaining oncotic pressure in the vascular system. The decrease in oncotic pressure due to low albumin levels causes fluid to leak from the interstitial spaces into the peritoneal cavity, creating multiple issues. Albumin is also very important for the transport of a variety of substances, such as drugs, lipids, hormones, and toxins that bind to albumin in the bloodstream. When drugs or other substances reach the liver, they are converted into water-soluble forms that can be separated from albumin and excreted, reducing toxicity.

Low serum albumin indicates poor liver function. The decrease in serum albumin levels is not seen in acute hepatic failure because it takes weeks of albumin production inhibition before serum albumin levels drop. The most common reason for low albumin is chronic hepatic failure due to cirrhosis; serum albumin levels are generally normal in chronic liver disease until cirrhosis and severe liver damage occur. In advanced liver disease, serum albumin levels may be less than 3.5 g/dL. Albumin levels can be low in conditions other than liver disease, such as severe malnutrition and some kidney diseases that cause widespread protein waste. Losses in albumin in urine due to kidney dysfunction (nephrotic syndrome) can lead to a decrease in serum albumin; albuminuria, excess albumin protein in urine, is a major sign of kidney pathology and preeclampsia. Severe burns that damage capillaries and blood vessels cause significant loss of serum protein; increased capillary permeability due to

burn damage causes persistent leakage of serum proteins in the vascular system.

When protein intake is inadequate, the body breaks down serum protein in the muscles and obtains sufficient amino acids for serum albumin synthesis. The US Department of Agriculture (USDA) recommends that women over 25 take 50 grams; more than 25 percent of their daily dietary protein consumption, about 65 grams. If a patient suffers from liver disease, the recommended dietary protein decreases according to the results of a liver function test, thereby reducing liver stress. Albumin levels do not fall from fasting or malnutrition until the condition becomes worse. The combination of long-term protein deficiency and serious disease is ultimately reflected in reduced serum albumin levels; there is no pathological condition that causes the liver to produce extra albumin. Thus, the increased rate is a reflection of dehydration: it plays a role of universality in the plasma that can bind to water, all kinds of cations, lipids, hormones, and so on. The most basic physiological bases known are osmotic pressure control, buffering and transporting of the blood as its most abundant protein is albumin. As mentioned above, it plays a major role in osmotic pressure control and transporting all kinds of substances in the blood, especially as a "taxi" of non-aqueous substances (including steroid-based hormones, metabolites, lipids, and drugs, for example). It is primarily produced in the liver.

XXI. Appendix

Report No. 1 to NTS Research

Evaluation of SAC Calcium for Tumor Growth Inhibition in the COLO 205 Human Colorectal Adenocarcinoma Nude Mouse Xenograft Model



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Report Reviewed By: Edgar Wood, Ph.D.

Date: June 7, 2017
Colo205-e347

CONFIDENTIAL

Charles River Discovery Services
Deliverables Revision

June 16, 2017

TO: Paul Lee, Ph.D.
NTS Research

RE: Revisions were made to the NTSR#01-Colo205-e347 Final Report dated June 7, 2017.

Revisions

Updated summary comments on Pages 1 and 10 based on client request and Research Director approval.

Impact

The overall outcome of the study was not impacted.

Action

The report has been formally amended, and this memo serves as clarification of the revisions stated above. The Acrobat® pdf file for the report will be amended to include this correction.

Executive Summary

NTS Research contracted Charles River Discovery Services North Carolina (CR Discovery Services) to evaluate SAC Calcium on two dosing schedules for tumor growth inhibition (TGI) in the COLO 205 human colorectal adenocarcinoma nude mouse xenograft model.

In the Colo205-e347 study, SAC Calcium was administered orally (p.o.) at 0.83 mg/kg to two groups. One received dosing twice daily with 1 dose on the first day (bid to end, first day 1 dose) and the other received dosing three times daily with 2 doses on the first day (tid to end, first day 2 doses). Treatments began on Day 1 in mice with established subcutaneous COLO 205 tumors. The study included a vehicle control group, dosed tid to end, first day 2 doses. The study endpoint was a mean tumor volume of 2000 mm^3 in the control group or 28 days, whichever came first. The study ended on Day 28. Treatment response was determined from an analysis of percent tumor growth inhibition (%TGI), defined as the percent difference between median tumor volumes (MTVs) of treated and control groups, with differences between groups deemed statistically significant at P 0.05 using the Mann-Whitney U test. Tumor regressions, mean tumor growth, and treatment tolerability also were considered.

In Summary, the median tumor volume on Day 28 for the control Group 1 was 936 mm^3. Treatment of animals with SAC Calcium at 0.83 mg/kg, administered bid to end, first day 1 dose and tid to end, first day 2 doses was associated with a difference ($P > 0.05$) TGI of 0% and 8%, respectively. Treatments administered in the study were acceptably tolerated based on group mean BW measurement and clinical observations.

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ABBREVIATIONS USED IN THIS REPORT:

BW	body weight
CR	complete regression
CR Discovery Services	CR Discovery Services North Carolina
DI	deionized
MTD	maximum tolerated dose
MTV	median tumor volume
NTR	non-treatment-related (death)
p.o.	oral(ly)
PR	partial regression
SAC Calcium	encoded WI1 for confidentiality
TGI	tumor growth inhibition
TR	treatment-related (death)
Vehicle	DI water

Introduction

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In the Colo205-e347 study, SAC Calcium was administered orally (p.o.) at 0.83 mg/kg to two groups. One received dosing twice daily with 1 dose on the first day (bid to end, first day 1 dose) and the other received dosing three times daily with 2 doses on the first day (tid to end, first day 2 doses). Treatments began on Day 1 in mice with established subcutaneous COLO 205 tumors. The study included a vehicle control group, dosed tid to end, first day 2 doses. The study endpoint was a mean tumor volume of 2000 mm^3 in the control group or 28 days, whichever came first. The study ended on Day 28. Treatment response was determined from an analysis of percent tumor growth inhibition (%TGI), defined as the percent difference between median tumor volumes (MTVs) of treated and control groups, with differences between groups deemed statistically significant at P 0.05 using the Mann-Whitney U test. Tumor regressions, mean tumor growth, and treatment tolerability also were considered. This report describes the methods, sampling, and results for the Colo205-e347 study.

Methods and Materials

Mice

Female NCI Ath/nu mice (Crl:NU(Ncr)-*Foxn1*^{nu}, Charles River) were ten weeks old and had a body weight (BW) range of 20.4 to 28.5 g on Day 1 of the study. The animals were fed *ad libitum* water (reverse osmosis, 1 ppm Cl) and NIH 31 Modified and Irradiated Lab Diet® consisting of 18.0% crude protein, 5.0% crude fat, and 5.0% crude fiber. The mice were housed on irradiated Enrich-o'cobs™ bedding in static microisolators on a 12-hour light cycle at 20–22 °C (68–72 °F) and 40–60% humidity. CR Discovery Services specifically complies with the recommendations of the *Guide for Care and Use of Laboratory Animals* with respect to restraint, husbandry, surgical procedures, feed and fluid regulation, and veterinary care. The animal care and use program at CR Discovery

Services is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, which assures compliance with accepted standards for the care and use of laboratory animals.

Tumor Cell Culture

Implantations were initiated from cultured COLO 205 human adenocarcinoma cells. Tumor cells were grown to mid-log phase in RPMI-1640 medium containing 10% fetal bovine serum, 100 units/mL penicillin G sodium, 100 g/mL streptomycin sulfate, 25 g/mL gentamicin, 2 mM glutamine, 1 mM sodium pyruvate, 10 mM HEPES and 0.075% sodium bicarbonate. Cell cultures were maintained in tissue culture flasks in a humidified incubator at 37 C, in an atmosphere of 5% CO_2 and 95% air.

***In Vivo* Implantation and Tumor Growth**

COLO 205 tumor cells were harvested during exponential growth and resuspended in PBS containing 50% Matrigel™ (BD Biosciences). On the day of tumor implant, each test mouse was injected subcutaneously in the right flank with 1 x 10^6 cells (0.1 mL cell suspension), and tumor growth was monitored as the average size approached the target range of 100–150 mm^3. Tumors were measured with calipers twice weekly in two dimensions. Tumor size was calculated using the formula:

$$\text{Tumor Volume } (\text{mm}^3) = \frac{w^2 \, l}{2}$$

where w = width and l = length, in mm, of a tumor. Tumor weight may be estimated with the assumption that 1 mg is equivalent to 1 mm^3 of tumor volume.

Nine days after tumor implantation, on Day 1 of the study, animals with individual tumor volumes ranging from 108 to 126 mm^3 were sorted into three groups (n = 10) with group mean tumor volumes of 115 mm^3.

Test Agents

NTS Research provided SAC Calcium (code-named WI1, Lot. No. SAC17001, received on 3/27/2017). CR Discovery Services assigned a code name for the purpose of

confidentiality during in-house testing. SAC Calcium was protected from light and stored at 25 ºC.

At the time of dosing, the SAC Calcium stock (8.383 mg/mL) was shaken well and diluted 1:100 with deionized (DI) water. The dosing suspension (0.8383 mg/mL) was mixed well and used within 0.5 hours.

Deionized (DI) water was used as vehicle in the study.

Treatment

On Day 1 of the study, mice bearing established COLO 205 tumors began dosing according to Table 1. Vehicle and SAC Calcium were administered p.o. in a dosing volume of 10 mL/kg; volumes were adjusted according to BW of the individual animals.

Group 1 animals served as controls and received DI water (vehicle), tid to end, first day 2 doses.

Group 2 animals received SAC Calcium at 0.83 mg/kg, bid to end, first day 1 dose.

Group 3 animals received SAC Calcium at 0.83 mg/kg, tid to end, first day 2 doses.

Endpoint and Tumor Growth Inhibition (TGI) Analysis

Tumors were measured using calipers twice per week. The study endpoint was defined as a mean tumor volume of 2000 mm^3 in the control group or 28 days, whichever came first. The study ended and tumor growth inhibition was calculated on Day 28.

The MTV (n), the median tumor volume for the number of animals, n, on the final day, was determined for each group. Percent tumor growth inhibition (%TGI) was defined as the difference between the MTV of the designated control group (Group 1) and the MTV of the drug-treated group, expressed as a percentage of the MTV of the control group:

$$\%\,TGI = \frac{MTV_{control} - MTV_{drug\text{-}treated}}{MTV_{control}} \times 100 = [1-(MTV_{drug\text{-}treated}/MTV_{control})] \times 100$$

The data set for TGI analysis includes all animals in a group, except those that die due to treatment-related (TR) or non-treatment-related (NTR) causes. CR Discovery Services

considers an agent that produces at least 60% TGI in this assay to be potentially therapeutically active.

Criteria for Regression Responses

Treatment efficacy may also be determined from the incidence and magnitude of regression responses observed during the study. Treatment may cause partial regression (PR) or complete regression (CR) of the tumor in an animal. In a PR response, the tumor volume was 50% or less of its Day 1 volume for three consecutive measurements during the course of the study, and equal to or greater than 13.5 mm^3 for one or more of these three measurements. In a CR response, the tumor volume was less than 13.5 mm^3 for three consecutive measurements during the course of the study. Animals were scored only once during the study for a PR or CR event and only as CR if both PR and CR criteria were satisfied. Animals were monitored for regression responses.

Toxicity

Animals were weighed daily for the first five days and then twice weekly. The mice were observed frequently for overt signs of any adverse, treatment-related (TR) side effects, and clinical signs were recorded when observed. Individual body weight was monitored as per protocol, and any animal with weight loss exceeding 30% for one measurement or exceeding 25% for three consecutive measurements was euthanized as a TR death (for treated groups). Group mean body weight loss was also monitored according to CR Discovery Services protocol. Acceptable toxicity was defined as a group mean body weight (BW) loss of less than 20% during the study and no more than 10% TR deaths. Any dosing regimen resulting in greater toxicity was considered above the maximum tolerated dose (MTD). Dosing was suspended in any group where mean weight loss exceeded acceptable limits. If group mean body weight recovered to acceptable levels, then dosing was modified to lower levels and/or reduced frequency then resumed. Deaths were classified as TR if it was attributable to treatment side effects as evidenced by clinical signs and/or necropsy. A TR classification was also assigned to deaths by unknown causes during the dosing period or within 14 days of the last dose. A death was classified as non-treatment-related (NTR) if there was no evidence that death was related to treatment side effects. NTR deaths are

further categorized as follows: NTRa describes deaths due to accidents or human error; NTRm is assigned to deaths thought to result from tumor dissemination by invasion and/or metastasis based on necropsy results; NTRu describes deaths of unknown causes that lack available evidence of death related to metastasis, tumor progression, accident or human error. It should be noted that treatment side effects cannot be excluded from deaths classified as NTRu.

Statistical and Graphical Analyses

Prism (GraphPad) for Windows 7.02 was used for graphical presentations and statistical analyses.

Statistical analyses of the differences between the Day 28 median tumor volumes (MTVs) of two groups were accomplished using the Mann-Whitney U test. Two-tailed statistical analyses were conducted at difference or significance level $P = 0.05$. Prism summarizes test results as difference, not significant (ns) at $P > 0.05$, significant (symbolized by "*") at $0.01 < P$ 0.05, very significant ("**") at $0.001 < P$ 0.01, and extremely significant ("***") at P 0.001. Because tests of statistical significance do not provide an estimate of the magnitude of the difference between groups, all levels of significance were described as either significant or not significant within the text of this report. Groups with regimens that exceeded the limits for acceptable toxicity were not evaluated statistically.

Box and whisker plots were constructed showing the Day 28 tumor volume data by group, with the "box" representing the 25th and 75th percentile of observations, the "line" representing the median of observations, and the "whiskers" representing the extreme observations. Group median tumor volumes were plotted as a function of time, and were truncated after the second TR death in a group. Percent group mean body weight changes from Day 1 were calculated for each group for each day of body weight measurement, and were plotted as a function of time. Error bars on the plots indicate one standard error of the mean (SEM).

Results

In the Colo205-e347 study, three groups of female nude mice were treated in accordance with Table 1. Table 2 presents a summary of the treatment responses. Figure 1 contains

box and whisker plots of the Day 28 tumor volume data used for TGI analysis. Figure 2 shows plots of group median tumor growth. Figure 3 presents plots of percent group mean body weight changes from Day 1. The raw data from the study, including clinical observations recorded during the study (Table A1), are located in Appendix A. Appendix B contains the detailed results of statistical analyses.

Efficacy

Growth of Colo205 Tumors in Control Mice (Group 1)

Group 1 mice received p.o. vehicle, tid to end, first day 2 doses, and served as the control group for calculation of percent TGI and statistical comparisons. Tumor growth in Group 1 was linear and progressive (Figure 2). The group had a median tumor volume on Day 28 of 936 mm^3 (n = 9) with individual tumor volumes ranging from 500 to 1470 mm^3 (Figure 1). One NTRu death occurred in the group on Day 14 (Table 1 and Table A1). Group 1 had no tumor regression responses or TR deaths.

Response to SAC Calcium Administered bid to end, first day 1 dose (Group 2)

Group 2 animals received SAC Calcium at 0.83 mg/kg, p.o., bid to end, first day 1 dose. Tumor growth in Group 2 closely paralleled that of control Group 1. Treatment resulted in a Day 28 median tumor volume of 936 mm^3 (n = 9), and a non-significant(-difference) 0% TGI relative to the vehicle control group ($P > 0.05$, Mann-Whitney). The group had one NTRu death on Day 24. Group 2 had no tumor regression responses or TR deaths.

Response to SAC Calcium Administered tid to end, first day 2 doses (Group 3)

Group 3 animals received SAC Calcium at 0.83 mg/kg, p.o., tid to end, first day 2 doses. Tumor growth in the group closely paralleled that seen for control animals. Treatment resulted in a Day 28 median tumor volume of 864 mm^3, corresponding to a difference 8% TGI relative to the vehicle control group ($P > 0.05$, Mann-Whitney).

Adverse Events

Figure 3 represents the percent mean BW changes from Day 1 for each group, and Table A1 documents Clinical Observations.

There were observations of tumor ulcerations in animals from all groups. There were no TR deaths in the study, and the only mean BW loss (Group 2, Day 2) was 0.4%.

Summary

The Colo205-e347 study evaluated SAC Calcium for tumor growth inhibition (TGI) in the COLO 205 human colorectal adenocarcinoma nude mouse xenograft model. Tumors were measured twice per week through Day 28, when TGI analysis was conducted.

In Summary, the median tumor volume on Day 28 for the control Group 1 was 936 mm^3. Treatment of animals with SAC Calcium at 0.83 mg/kg, administered bid to end, first day 1 dose and tid to end, first day 2 doses was associated with difference ($P > 0.05$) TGI of 0% and 8%, respectively. Treatments administered in the study were acceptably tolerated based on group mean BW measurement and clinical observations.

Table 1

Protocol Design for the Colo205-e347 Study

Group	n	Treatment Regimen			
		Agent	mg/kg	Route	Schedule
1	10	vehicle	---	po	tid to end first Day 2 doses
2	10	SAC Calcium	0.83	po	bid to end first Day 1 dose
3	10	SAC Calcium	0.83	po	tid to end first Day 2 doses

Table 1 displays the study design as of Day 1 of the study.
vehicle = DI Water

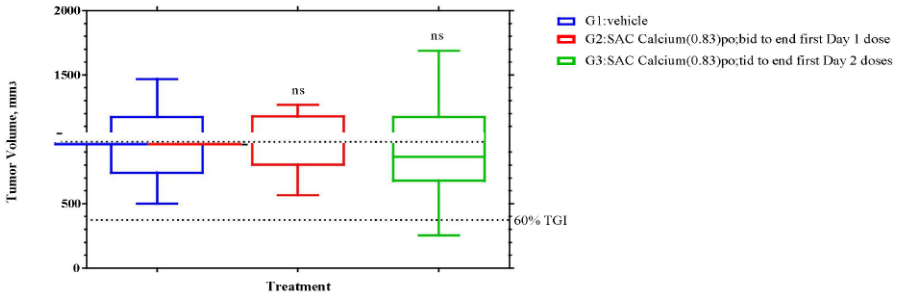
Table 2

Tumor Growth Inhibition in the Colo205-e347 Study

Group	n	Treatment Regimen				MTV (n)		Statistical	Regressions			Mean BW	Deaths	
		Agent	mg/kg	Route	Schedule	Day 28	%TGI	Significance	PR	CR	TFS	Nadir	TR	NTR
1	9	vehicle	---	po	tid to end first Day 2 doses	936 (9)	---	---	0	0	0	---	0	1
2	9	SAC Calcium	0.83	po	bid to end first Day 1 dose	936 (9)	0	ns	0	0	0	-0.4% Day 2	0	1
3	10	SAC Calcium	0.83	po	tid to end first Day 2 doses	864 (10)	8	ns	-	-	-	---	0	0

Table 2 displays the final treatment regimen at completion of the study.
vehicle = DI Water
Study Endpoint = 2000 mm³; Study Duration = 28 Days
n = number of animals in a group not dead from accidental or unknown causes, or euthanized for sampling
%TGI = $[1-(MTV_{drug\ treated}/MTV_{control})] \times 100$ = percent tumor growth inhibition, compared to Group 1
Statistical Significance (Mann-Whitney *U* test): ne = not evaluable, ns = not significant, * = $P \leq 0.05$, *** = $P \leq 0.01$, **** = $P \leq 0.001$, compared to Group 1
MTV (n) = median tumor volume (mm³) for the number of animals on the Day of TGI analysis (includes animals with tumor volume at endpoint)
PR = partial regression; **CR** = complete regression; **TFS** = tumor free survivors, i.e., CRs at end of study
Mean BW Nadir = lowest group mean body weight, as % change from Day 1; --- indicates no decrease in mean body weight was observed **TR** = treatment-related death; **NTR** = non-treatment-related death

Figure 1. Tumor Volume Distribution on Day 28 in Colo205-e347



Statistical Significance for Kruskal Wallis Dunn's or Mann-Whitney *U* test: ns = non-significant, * = $0.01 < P \leq 0.05$, ** = $0.001 < P \leq 0.01$, *** = $P \leq 0.001$ when compared to Group 1.
TGI $\geq$ 60% indicates potential therapeutic activity.

Figure 2. Median Tumor Volume in Colo205-e347

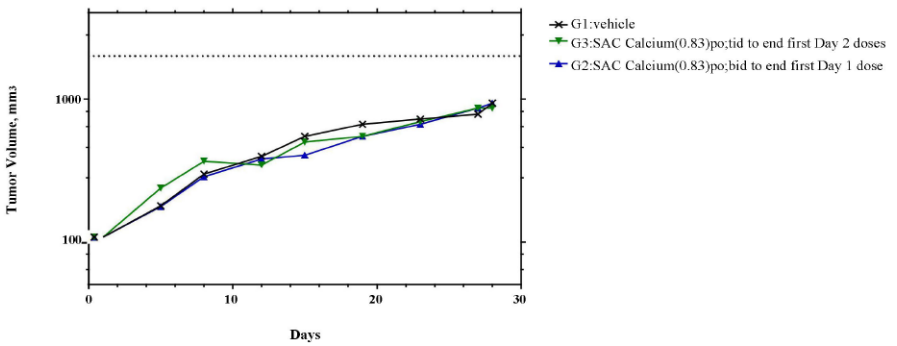
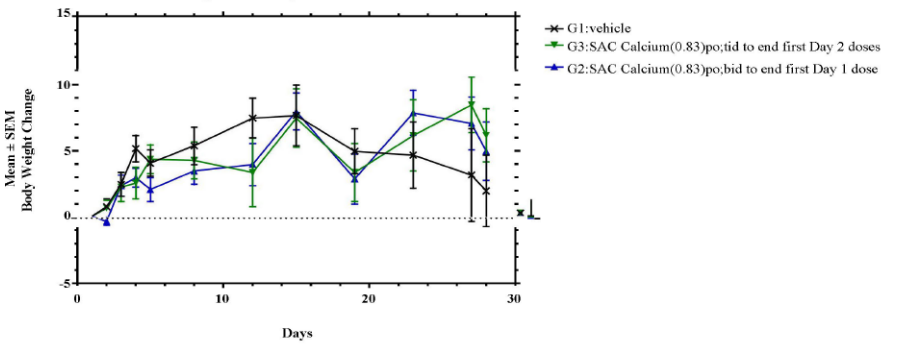


Figure 3. Percent Group Mean Body Weight Changes from Day 1 in Colo205-e347



APPENDIX A

Raw Data
(Colo205-e347)

Abbreviations (not all abbreviations may appear in this report):

A#	Animal number
BW	Body Weight
CM	Client Manager
CR	Charles River (vendor)
CR	Complete regression (responder)
DSS	Dead on survival study
ES	Euthanized for sampling
Gr.	Group
ip	intraperitoneal
itu	intratumor
iv	intravenous
L	length (caliper)
mm	millimeter
MTD	Maximum tolerated dose
NTRa	Non-treatment-related death due to accident or error
NTRm	Non-treatment-related death due to metastasis
NTRu	Non-treatment-related death due to unknown etiology
po	per os, oral
PR	Partial regression (responder)
RA	Research Associate (Technician)
sc	subcutaneous
SD	Study Director
SEM	Standard Error of the Mean
STDEV	Standard Deviation
TFS	Tumor free survivor
TGD	Tumor Growth Delay
TGI	Tumor Growth Inhibition
TR	Treatment-related death
TP	Tumor Progression
TV	Tumor Volume
W	width (caliper)

Color indicators in raw data:

Body Weight	Italicized red font	BW decrease of ≥ 15% from Day 1
Caliper Measurement	Dark blue shading	Double tumor present
Tumor Volume	Italicized red font	PR criteria satisfied
Tumor Volume	Bold red font	CR criteria satisfied

Confidential

Study: Colo205-e347 (Started 03/30/17)
Study Type: TGI
Client: NTSR
RA: Rhiannon Roark
SD: Elizabeth Rainbolt
CM: Anna Avrutskaya

Drugs and Treatment:

Gr.	N	Regimen 1			
		Agent	mg/kg	Route	Schedule
1#	10	vehicle	---	po	tid to end first Day 2 doses
2	10	SAC Calcium	0.83	po	bid to end first Day 1 dose
3	10	SAC Calcium	0.83	po	tid to end first Day 2 doses

- Control Group

Animals

Age on Day 1:	10 weeks	**Implant Date:**	3/21/2017
Location:	BioBubble C	**Implant Source:**	$1*10^6$ Colo205 (04) tumor cells in 50% Matrigel
Vendor:	CR	**Implant to Start:**	9 Days
Species:	Mouse	**Group Mean Target Range:**	100 to 150 mg
Strain:	NCI Ath/nu	**1st Measurement Day Individual Tumor Range:**	Min - 108 mm^3, Max - 126 mm^3 (Day 1)
Gender:	female	**1st Measurement Day Group Mean Tumor Range:**	Min - 115.2 mm^3, Max - 115.2 mm^3 (Day 1)
Shipment:	A-16-1839-CR (02/15/2017)	**BW Range:**	Min - 20.4 g, Max - 28.5 g
Endpoint Conditions:	mean TV of 2000 mg in control or 28 Days		

Compound Information

Client Compound Name	PRC Code	Active %	Lot #	Received Date	Shipment #
SAC Calcium	WI	100	SAC17001	3/27/2017	C-17-0001-NTSR

Experiment Number: Colo205-e347 The Experiments Started on: 03/30/17

Body Weight

Group 1: vehicle (po, tid to end first Day 2 doses)

Date	03/30/17	03/31/17	04/01/17	04/02/17	04/03/17	04/06/17	04/10/17	04/13/17	04/17/17	04/21/17	04/25/17	04/26/17
Day of Study	1	2	3	4	5	8	12	15	19	23	27	28
A#	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)
1	23.50	23.60	24.20	24.80	24.40	24.60	24.70	25.00	25.20	25.50	25.40	25.10
2	22.50	22.50	23.20	24.20	23.80	23.60	23.90	24.00	23.20	23.10	*17.30*	*18.80*
3	27.30	26.90	26.80	27.30	27.30	26.80	27.90	28.60	29.00	28.00	27.80	27.70
4	21.20	22.20	22.80	23.50	23.40	23.80	23.50	24.10	22.80	23.60	22.60	21.90
5	23.30	23.60	24.30	23.90	23.80	24.10	24.40	24.00	23.30	21.40	24.10	23.80
6	25.20	25.30	25.70	26.40	25.90	26.00	26.90	27.40	25.60	26.80	26.90	26.20
7	24.00	23.90	24.40	24.70	24.00	23.50	24.00	NTRn on 04/12/17				
8	22.80	22.50	22.80	24.20	23.70	24.40	26.30	26.30	25.60	25.40	25.50	24.80
9	21.70	21.90	22.10	22.40	22.20	22.70	22.30	20.40	20.90	20.50	21.00	20.60
10	24.90	25.30	25.60	26.60	26.30	27.60	28.20	28.70	27.20	27.90	28.10	27.50
Mean	**23.6**	**23.8**	**24.2**	**24.8**	**24.5**	**24.7**	**25.2**	**25.4**	**24.8**	**24.7**	**24.3**	**24**
STDEV	**1.8**	**1.6**	**1.5**	**1.5**	**1.5**	**1.6**	**2**	**2.7**	**2.5**	**2.7**	**3.5**	**3.1**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**9**	**9**	**9**	**9**	**9**

Group 2: SAC Calcium (0.83 mg/kg,po, bid to end first Day 1 dose)

Date	03/30/17	03/31/17	04/01/17	04/02/17	04/03/17	04/06/17	04/10/17	04/13/17	04/17/17	04/21/17	04/25/17	04/26/17
Day of Study	1	2	3	4	5	8	12	15	19	23	27	28
A#	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)
1	24.60	24.80	24.30	24.90	24.50	25.20	25.00	26.40	25.10	27.40	26.70	26.50
2	22.00	22.10	22.40	22.70	22.60	23.20	23.70	24.50	23.70	24.60	24.10	23.50
3	20.90	20.90	22.20	22.40	22.40	22.60	23.10	23.50	21.90	21.90	22.00	21.20
4	23.40	23.30	24.00	24.10	23.20	24.20	25.00	25.10	22.90	24.70	23.70	23.10
5	24.40	24.50	24.80	25.70	25.50	26.60	25.20	25.70	24.50	23.70	NTRn on 04/22/17	
6	24.80	24.50	24.90	24.60	24.60	24.50	24.60	25.90	25.10	25.30	25.10	25.00
7	27.20	26.90	28.80	27.90	28.00	27.70	26.40	27.90	25.10	27.60	27.60	26.20
8	28.50	27.90	28.80	29.60	29.40	29.90	31.10	32.70	31.50	33.40	34.30	34.00
9	26.40	26.60	27.00	27.20	26.80	26.50	26.60	27.60	27.00	28.00	28.10	27.80
10	21.50	21.40	22.00	22.30	22.20	23.00	22.40	23.10	23.10	23.50	23.10	22.80
Mean	**24.4**	**24.3**	**24.9**	**25.1**	**24.9**	**25.3**	**25.3**	**26.2**	**25**	**26**	**26.1**	**25.6**
STDEV	**2.5**	**2.4**	**2.5**	**2.5**	**2.5**	**2.3**	**2.4**	**2.8**	**2.7**	**3.3**	**3.7**	**3.8**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**9**	**9**

Group 3: SAC Calcium (0.83 mg/kg,po, tid to end first Day 2 doses)

Date	03/30/17	03/31/17	04/01/17	04/02/17	04/03/17	04/06/17	04/10/17	04/13/17	04/17/17	04/21/17	04/25/17	04/26/17
Day of Study	1	2	3	4	5	8	12	15	19	23	27	28
A#	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)	Wt (g)
1	22.50	23.00	23.00	23.00	23.20	23.90	25.00	25.60	23.70	25.00	24.90	23.50
2	20.40	20.80	21.00	21.20	21.50	22.20	23.10	23.30	21.80	22.20	23.40	23.30
3	24.60	24.10	24.20	24.70	24.90	24.70	26.00	26.60	26.70	26.70	26.30	25.30
4	25.10	24.60	24.50	24.90	25.20	25.10	25.10	24.80	24.70	25.10	25.40	25.10
5	24.10	24.90	26.20	26.30	26.00	26.30	27.30	27.50	26.40	26.90	27.30	26.80
6	23.40	23.60	23.30	24.00	23.90	24.20	22.80	25.00	24.70	25.70	25.00	25.00
7	21.30	21.30	21.50	22.70	22.50	21.80	21.30	21.80	21.40	22.70	22.50	21.80
8	23.80	23.60	24.80	25.00	24.80	24.00	21.20	24.60	23.20	25.60	26.00	25.50
9	26.10	26.60	27.80	25.10	29.20	29.20	28.20	30.30	29.20	29.40	31.10	30.30
10	23.10	23.40	23.20	23.40	23.30	22.90	22.10	22.20	20.40	*19.50*	22.20	22.20
Mean	**23.4**	**23.6**	**24**	**24**	**24.5**	**24.4**	**24.2**	**25.2**	**24.2**	**24.9**	**25.4**	**24.9**
STDEV	**1.7**	**1.7**	**2**	**1.5**	**2.1**	**2.1**	**2.5**	**2.5**	**2.7**	**2.8**	**2.6**	**2.5**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Experiment Number: Colo205-a547 The Experiments Started on: 03/30/17

Caliper Measurement

Group 1: vehicle (po, bid to end first Day 2 doses)

Date	03/30/17		04/03/17		04/06/17		04/10/17		04/13/17		04/17/17		04/21/17		04/25/17		04/26/17	
Day of Study	1		5		8		12		15		19		23		27		28	
A#	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)
1	5	6	6	7	7	7	7	8	9	10	9	11	11	12	12	14	12	14
2	5	6	7	8	9	9	10	10	11	12	12	13	13	14	10	11	10	12
3	5	6	7	7	8	8	8	9	10	10	11	11	12	13	13	13	13	14
4	5	6	7	7	7	8	8	8	10	10	10	11	12	13	13	14	13	14
5	5	6	7	8	8	9	9	10	10	11	10	12	9	10	10	10	10	10
6	5	6	7	8	8	9	9	10	10	11	9	10	11	11	11	12	12	13
7	5	7	8	9	9	11	10	11	NTRu on 04/12/17									
8	5	7	7	8	8	9	9	10	11	11	12	12	13	13	14	14	14	15
9	5	7	7	8	8	9	9	10	10	11	11	11	10	11	11	13	12	13
10	5	7	8	8	9	10	9	10	11	11	11	11	11	11	11	12	12	12

Group 2: SAC Calcium (0.83 mg/kg,po, bid to end first Day 1 dose)

Date	03/30/17		04/03/17		04/06/17		04/10/17		04/13/17		04/17/17		04/21/17		04/25/17		04/26/17	
Day of Study	1		5		8		12		15		19		23		27		28	
A#	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)
1	5	6	7	7	8	9	9	9	10	11	11	12	12	13	12	15	12	17
2	5	6	6	7	6	10	8	10	9	11	10	12	11	13	12	16	12	16
3	5	6	6	7	8	8	8	9	9	10	10	11	11	12	12	14	12	14
4	5	6	7	8	8	8	9	10	10	11	10	11	11	11	11	12	12	12
5	5	6	6	8	7	10	8	11	9	12	10	13	11	13	NTRu on 04/22/17			
6	5	6	7	7	8	8	9	10	8	8	8	9	11	11	12	12	12	13
7	5	7	7	8	9	9	10	11	10	11	10	11	11	11	12	12	12	13
8	5	7	7	8	7	8	6	6	6	7	7	9	9	11	10	13	11	12
9	5	7	6	9	7	11	7	11	7	12	8	13	8	13	9	13	9	14
10	5	7	7	9	8	11	9	12	8	9	9	10	10	11	12	14	13	15

Group 3: SAC Calcium (0.83 mg/kg,po, tid to end first Day 2 doses)

Date	03/30/17		04/03/17		04/06/17		04/10/17		04/13/17		04/17/17		04/21/17		04/25/17		04/26/17	
Day of Study	1		5		8		12		15		19		23		27		28	
A#	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)
1	5	6	7	8	8	9	10	11	10	10	11	12	12	12	12	12	12	13
2	5	6	8	9	9	10	11	11	12	12	13	15	15	15	13	14	13	14
3	5	6	7	7	8	8	9	9	10	10	10	10	10	11	11	12	11	12
4	5	6	7	7	7	8	8	10	11	11	10	10	11	12	12	12	12	12
5	5	6	7	8	8	9	9	10	10	10	10	11	11	11	10	10	10	10
6	5	6	8	9	9	10	8	8	9	10	12	12	13	14	14	15	15	15
7	5	7	8	10	9	10	9	9	9	9	10	10	11	12	12	13	13	14
8	5	7	7	9	8	10	7	8	7	7	8	8	9	11	11	12	12	12
9	5	7	8	9	9	10	8	9	10	10	10	11	10	12	12	12	12	12
10	5	7	8	9	9	10	10	12	10	12	11	13	8	8	7	8	8	8

Experiment Number: Colo205-e347 The Experiments Started on: 03/30/17

Tumor Volume

Group 1: vehicle (po, tid to end first Day 2 doses)

Date	03/30/17	04/03/17	04/06/17	04/10/17	04/13/17	04/17/17	04/21/17	04/25/17	04/26/17
Day of Study	1	5	8	12	15	19	23	27	28
A#	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)
1	108	127	173	210	405	446	726	1008	1008
2	108	220	351	491	726	936	1183	550	600
3	108	162	242	312	500	666	936	1099	1183
4	108	179	209	288	500	550	936	1183	1183
5	108	202	306	398	550	600	405	500	500
6	108	170	298	400	550	405	666	726	936
7	126	299	441	560	NTRu on 04/12/17				
8	126	165	301	385	666	864	1099	1372	1470
9	126	209	292	455	550	666	550	787	936
10	126	235	357	462	666	666	666	726	864
Mean	**115.2**	**196.8**	**297.1**	**396.2**	**568**	**644.1**	**796.2**	**883.3**	**964.4**
SEM	**2.9**	**15.1**	**24.4**	**32.9**	**33.5**	**58**	**86**	**99.1**	**99.5**
n	**10**	**10**	**10**	**10**	**9**	**9**	9	9	9

Group 2: SAC Calcium (0.83 mg/kg.po, bid to end first Day 1 dose)

Date	03/30/17	04/03/17	04/06/17	04/10/17	04/13/17	04/17/17	04/21/17	04/25/17	04/26/17
Day of Study	1	5	8	12	15	19	23	27	28
A#	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)
1	108	189	295	420	550	726	936	1080	1224
2	108	116	199	296	446	600	787	1152	1152
3	108	138	248	282	405	550	726	1008	1008
4	108	176	294	436	550	550	666	726	864
5	108	153	263	366	486	650	787	NTRu on 04/22/17	
6	108	177	286	380	256	288	666	864	936
7	126	223	351	532	550	550	666	864	936
8	126	219	200	112	126	221	446	650	726
9	126	171	254	246	294	416	416	527	567
10	126	197	327	466	288	405	550	1008	1268
Mean	**115.2**	**176**	**271.7**	**353.6**	**395.1**	**495.6**	**664.3**	**875.4**	**964.5**
SEM	**2.9**	**10.7**	**15.5**	**38.7**	**46.7**	**50.6**	**50.6**	**69.6**	**76.6**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	9	9

Group 3: SAC Calcium (0.83 mg/kg.po, tid to end first Day 2 doses)

Date	03/30/17	04/03/17	04/06/17	04/10/17	04/13/17	04/17/17	04/21/17	04/25/17	04/26/17
Day of Study	1	5	8	12	15	19	23	27	28
A#	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)	TV (mm^3)
1	108	207	316	501	500	726	864	864	936
2	108	269	427	604	864	1268	1688	1183	1183
3	108	161	239	337	500	500	550	726	726
4	108	167	216	355	666	500	726	864	864
5	108	218	268	385	500	550	666	500	500
6	108	250	389	271	405	864	1183	1470	1688
7	126	303	406	330	365	500	726	936	1183
8	126	232	347	180	172	256	446	726	864
9	126	243	419	271	500	550	600	864	864
10	126	259	392	558	600	787	256	196	256
Mean	**115.2**	**231**	**341.8**	**379.1**	**507.1**	**650**	**770.4**	**832.9**	**906.4**
SEM	**2.9**	**14**	**24.6**	**42.9**	**58.2**	**87.8**	**128.4**	**109.6**	**124.3**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table A1. Colo205-e347 Clinical Observations

Group	Animals	Date	Day	Clinical Observations
1	2	4/24/2017	26	Very thin; hunched; pale; 17 grams. (AUS) Afternoon re-check; warm and active; placed food on floor. (LU)
1	5	4/20/2017	22	Slightly pale; moderate tumor ulceration; warm and active. (RR)
1	7	4/10/2017	12	Mild scabbing on tumor; active and alert. (RR)
1	7	4/12/2017	14	Animal cool to touch; pale; lethargic; increased respiratory effort; tumor severely cannibalized; open wound on flank where tumor was; 2 grams in body weight loss. (RR) Euthanized; Necropsy: no abnormalities found internally; tumor site appeared the same as noted above; NTRu. (KMS)
1	9	4/13/2017	15	Mild tumor ulceration; animal mildly pale; warm; active. (RR)
1	9	4/21/2017	23	Moderate tumor ulceration. (RR)
1	10	4/10/2017	12	Mild tumor ulceration; active and alert. (RR)
1	10	4/13/2017	15	Small amount of blood coming from tumor; active, alert. (DS)
2	5, 8	4/10/2017	12	Mild tumor ulceration; active and alert. (RR)
2	5	4/22/2017	24	Found dead; beyond necropsy; cannibalized; NTRu. (AM)
2	8	4/5/2017	7	Mild tumor ulceration; active and alert. (RR)
2	10	4/13/2017	15	Mild tumor ulceration. (RR)
3	1, 6, 7, 9	4/13/2017	15	Mild tumor ulceration. (RR)
3	2	4/25/2017	27	Soft 15 x 16 mass on back of animal between shoulder blades; active and alert. (RR)
3	6, 7, 9	4/10/2017	12	Mild tumor ulceration; active and alert. (RR)
3	8	4/10/2017	12	Animal slightly pale; warm and active; 3 grams body weight loss; mild tumor ulceration. (RR)
3	10	4/15/2017	17	Animal slightly pale; active and alert. (RB)
3	10	4/18/2017	20	Animal severely pale and hunched; cold to touch; tumor severely ulcerated; shallow breathing observed. (RR) Re-check: moderately pale; cool to touch; active. (MRCT)

Table A2. Confirmed Dosing for Colo205-e347

Treatments					Volume mL	Dosing	THU	FRI	SAT	SUN	MON	TUE	WED	THU	FRI	SAT	SUN	MON	TUE	WED	THU	FRI	SAT	SUN	MON	TUE	WED
							03/30/17	03/31/17	04/01/17	04/02/17	04/03/17	04/04/17	04/05/17	04/06/17	04/07/17	04/08/17	04/09/17	04/10/17	04/11/17	04/12/17	04/13/17	04/14/17	04/15/17	04/16/17	04/17/17	04/18/17	04/19/17	04/20/17	04/21/17	04/22/17	04/23/17	04/24/17	04/25/17	04/26/17
Group #	Drug	mg/kg	Route	Schedule	mL		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
							Doses per Day																											
Group 1#	vehicle	---	po	tid to end first Day 2 doses	0.2		2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1
Group 2	SAC Calcium	0.83	po	bid to end first Day 1 dose	0.2		1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1
Group 3	SAC Calcium	0.83	po	tid to end first Day 2 doses	0.2		2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1

- Control Group

APPENDIX B

Statistical Analyses
(Colo205-e347)

Groups Compared	vehicle	vehicle
	Group 1 vs 2	**Group 1 vs 3**
	SAC Calcium(0.83) (po)bid to end first Day 1 dose	SAC Calcium(0.83) (po)tid to end first Day 2 doses
Mann-Whitney U-test: D28 TV		
P Value	0.9805	0.5324
Exact or approximate P value?	Exact	Exact
P value summary	ns	ns
Are medians signif. diff.?(P<0.05)	No	No
One- or two-tailed P value?	Two-tailed	Two-tailed
Sum of ranks in column A,B	85 , 86	98 , 92
Mann-Whitney U	40	37
Difference between medians		
Median of Column A	936, n=9	936, n=9
Median of Column B	936, n=9	864, n=10
Difference: Actual	0	-72
Difference: Hodges-Lehmann	0	-72

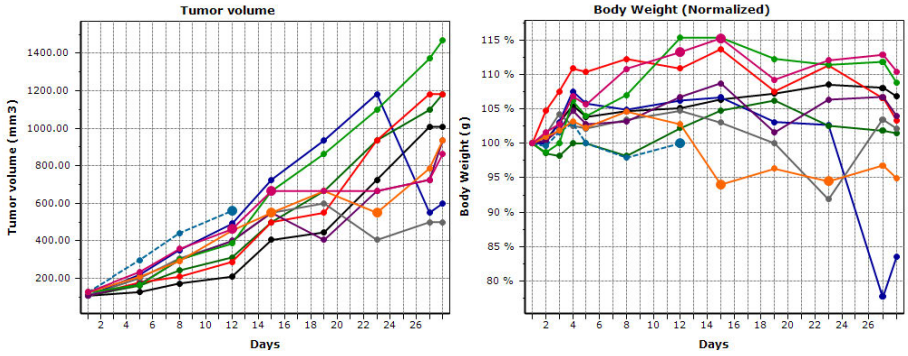
ns = non-significant, $P > 0.05$

* = $P \leq 0.05$

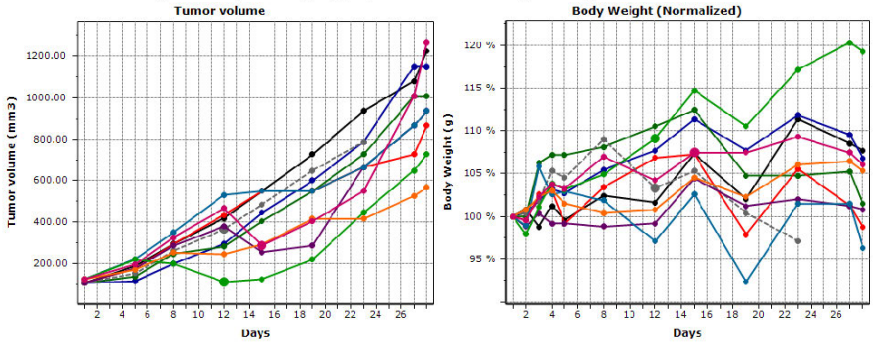
** = $P \leq 0.01$

*** = $P \leq 0.001$

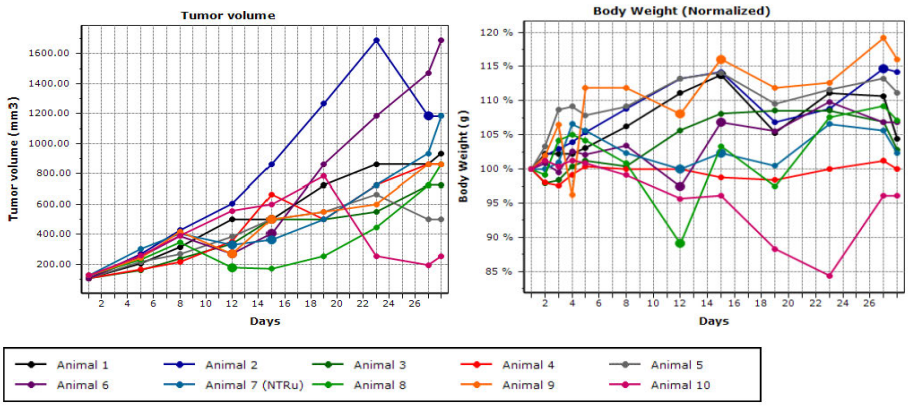
Colo205-e347 Group 1: vehicle (po, tid to end first day 2 doses)



Colo205-e347 Group 2: WI1 (0.83 mg/kg,po, bid to end first day 1 dose)



Colo205-e347 Group 3: WI1 (0.83 mg/kg,po, tid to end first day 2 doses)



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Effects of Sigma Anti-bonding Molecule Calcium Carbonate on bone turnover and calcium balance in ovariectomized rats

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This study was conducted to evaluate the effect of Sigma Anti-bonding Molecule Calcium Carbonate (SAC) as therapy for ovariectomy-induced osteoporosis in rats. Three weeks after surgery, fifteen ovariectomized Sprague-Dawley rats were divided randomly into 3 groups: sham-operated group (sham), ovariectomized group (OVX) and SAC-treatment group (OVX+SAC). The OVX+SAC group was given drinking water containing 0.0012% SAC for 12 weeks. Bone breaking force and mineralization as well as blood parameters related to the bone metabolism were analyzed. In OVX animals, blood concentration of 17β-estradiol decreased significantly, while osteocalcin and type I collagen C-terminal telopeptides (CTx) increased. Breaking force, bone mineral density (BMD), calcium and phosphorus in femurs, as well as uterine and vaginal weights, decreased significantly following OVX. However, SAC treatment (0.0012% in drinking water) not only remarkably restored the decreased 17β-estradiol and increased osteocalcin and CTx concentrations, but also recovered decreased femoral breaking force, BMD, calcium and phosphorus, although it did not reversed reproductive organ weights. It is suggested that SAC effectively improve bone density by preventing bone turnover mediated osteocalcin, CTx and minerals, and that it could be a potential candidate for therapy or prevention of menopausal osteoporosis.

Key words: Osteoporosis, bone mineral density, Sigma Anti-bonding Molecule Calcium Carbonate, 17β-estradiol, osteocalcin, type I collagen C-terminal telopeptides

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Osteoporosis is defined pathologically as a skeletal disorder characterized by absolute decrease in the amount of bone, leading to fractures after minimal trauma [1]. Also, osteoporosis is a major age-related health problem for men and women who often have a negative calcium balance due to decreased intestinal calcium absorption, insufficient dietary calcium intake, as well as increased urinary Ca loss associated with estrogen deficiency during menopause [2]. The main underlying cause of fractures in osteoporosis is increased bone fragility as a result of bone loss. Fracture risk is determined by absolute bone mineral density (BMD), regardless of age [3-6]. Low BMD is realized as the most important risk factor that can lead to osteoporosis [7,8].

In humans, osteoporosis was classified as 3 types; postmenopausal osteoporosis (Type I), age-related osteoporosis (Type II) and secondary osteoporosis (Type III). Postmenopausal osteoporosis typically affects women by estrogen deficiency within 15 to 20 years after menopause [9,10]. BMD of bilateral ovariectomized women is significantly lower than similarly aged women with normal ovarian function [11]; 50-year-old women who were ovariectomized at about 30 years old have similar bone density as 70-year-old women who become menopausal at the age of 50. This fact indicates that the decline of ovarian function is more important than age [12]. Therefore, postmenopausal administration of estrogen decreases the occurrence of fractures associated with

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osteoporosis by about one half [1].

In patients with osteoporosis, estimation of biochemical markers indicating bone turnover and bone quality has been known to be useful in predicting the rates of bone loss in postmenopausal women [13-15]. Bone quality is determined by structural and material properties that are influenced by bone turnover rate [16]. Histomorphometry is a method of estimating method of bone quality in addition to newer imaging techniques such as microcomputed tomography and magnetic resonance microimaging of bone non-invasively, but the use of these techniques is restricted due to their limited availability, high cost and relatively high radiation exposure [16]. Accelerated bone turnover leads to the irreversible loss of some trabeculae, resulting in weaker bone and increased risk of fracture [16]. Therefore, bone turnover is most commonly assessed in clinical practice by the measurement of biochemical markers of bone turnover. Detectable bone turnover markers are products of osteoblast/osteocyst and osteoclast type I collagens (CTx) breakdown reflecting bone formation and bone resorption, respectively [13,16]. CTx, and bone type I collagen C-telopeptides, are products of collagen degradation and therefore mirrors bone resorption; elevated CTx levels generally mean accelerated bone turnover [16]. Therefore, estimation of BMD and CTx may be helpful for predicting fracture risks.

Drugs used in the treatment of osteoporosis can be classified in two groups; antiresorptive or stimulators of bone formation. Antiresorptive drugs include calcium, estrogen, calcitonin and bisphosphonates [17-20]. Plant estrogen for osteoporosis treatment has been used for prevention of bone resorption [21]. But, discontinuation of estrogen administration leads to the recurrence of bone resorption and the degree is similar with bone resorption after ovariectomy. Also, severe side effects of estrogen treatment are endometrial carcinoma, venous thrombosis and pulmonary embolism [1]. Bisphosphonates are generally well tolerated, with few side effects, which include gastrointestinal symptoms such as nausea, vomiting and diarrhea. However, recent findings suggest that bisphosphonates induce osteonecrosis of the jaw, atrial fibrillation, acute phase response and renal insufficiency, raising safety and ethical concerns around the use of bisphosphonates [20]. Therefore, new drugs need development for use in the prevention of osteoporosis, particularly in postmenopausal women.

Sigma Anti-bonding Molecule Calcium Carbonate (SAC; activated ionic calcium) harvested from oyster shells as the raw material, results in production of calcium oxide; this calcium has a weak bonding force with other molecules, radicals or atoms. Therefore, this treated calcium can easily be absorbed into the cell, increasing an animal's metabolic system. Also, SAC helps growth and increases endogenous growth hormone by effectively supplying calcium into the cell which is needed for metabolism.

Therefore, this study was performed to evaluate the effect of SAC as a therapy for osteoporosis in an osteoporotic rat model induced by ovariectomy. Also, we investigated the effects of the SAC on BMD, bone and mineral metabolism as well as biochemical markers of bone turnover in osteoporotic rats.

Materials and Methods

Chemicals

SAC was obtained from NTS Research & Inc. (Coquitlam, BC, Canada). The composition of SAC (above 98% in purity) is shown in Table 1. Specific gravity (at 20°C) was 7.0-7.5 and heavy metals (As, Pb, Cd and Hg) were <1 ppm.

Animals

Twelve-week old female SPF Sprague-Dawley rats (250 g body weight; n=15) were purchased from the Daehan Laboratory Animal Center (Eumseong, Korea). They were housed in stainless net cages (800W×900L×650H mm) or fenced rooms (1,300W×3,720L×1,250H mm) and 5 rats were contained in each place. The housing conditions as follows: temperature, 23±3°C; relative humidity, 55±10%; lighting time, 12 h (08:00-20:00); ventilation frequency, 10-20 changes/h; illuminance, 150-300 lux. The temperature and humidity was measured hourly by a thermo-hygrometer and there were no variables affecting results of the experiments. The animals were fed a standard rodent chow and purified water *ad libitum*. All experimental procedures were approved and carried out in accordance with the Institutional Animal

Table 1. Composition of Sigma Anti-bonding Molecule Calcium Carbonate

Contents	Concentration (weight %)
$CaCO_3$	98.5
Na_2O	0.58
MgO	0.49
SiO_2	0.22
Fe_2O_3	0.02
K_2O	0.04
MnO_2	0.001
TiO_2	0.002
Al_2O_3	0.012
Cl	0.02
S	0.09
P	trace
Cu	trace
Ni	trace

Care and Use Committee of Laboratory Animal Research Center at Chungbuk National University, Korea.

Grouping and treatment

Experimental animals were divided randomly into 3 groups of 5 female SD rats. The acclimatized rats underwent either bilateral laparotomy (sham, n=5) or bilateral ovariectomy (OVX, n=10). Three weeks after recovering from surgery, the OVX rats were randomly divided into two groups: vehicle-treated (OVX, n=5) and SAC-treated (OVX+SAC, n=5). Only OVX+SAC rats were given drinking water containing 0.0012% SAC for 12 weeks following 3 weeks post-operation. Changes in body weight and food and water intakes were measured at a regular time. The food and water intakes were calculated by differences between previous intake and the daily measured value.

Blood collection

Blood samples were collected 12 weeks after surgery. Before the blood collection, animals were fasted for 16 h and anesthesia (Zoletile 50®, Virbac, Korea) was given. After an incision in the inguinal region, 0.5 mL of blood was collected from the abdominal artery and kept in a refrigerator (4°C). A part of the blood was used for hematology. Remaining blood was centrifuged at 3,000 rpm for 10 min to obtain serum. The serum was used for blood biochemistry, and for analyses of 17β-estradiol, osteocalcin and CTx which are bone metabolism indicators.

Hematology and blood biochemistry

Hematologic tests were performed using T-540 Coulter Counter (Coulter Electronics Inc, Hialeah, USA) and ADVIA120 Hematology System (Bayer Healthcare LLC, Tarrytown, USA). White blood cells (WBC), differential leukocyte counts, hematocrit, red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets were analyzed.

The following parameters were analyzed for blood biochemistry (Ciba-Corning 644 Na/K/Cl Analyzer, Ciba-Corning, Medfield, USA): alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), glucose, triglycerides (TG), total cholesterol (TC), high-density lipoproteins (HDL), low-density lipoproteins (LDL), total proteins (TP), albumin, calcium and phosphorus. As parameters of bone metabolism, serum 17β-Estradiol concentrations were analyzed using a 17β-estradiol kit (Coat-A-Count® Estradiol: Diagnostic Products Corporation, Los Angeles, USA). Osteocalcin levels were determined using a sandwich ELISA kit (Biomedical Technologies Inc., Stoughton, USA), and CTx levels were measured using a serum RatLaps ELISA kit (IDS Inc., Fountain Hills, USA).

Measurement of bone density and minerals

Bone density of the femur was measured using dual energy X-ray absorptionmetry (DEXA; Prodigy Advance, Donga imaging, Korea). Just after BMD measurement, mechanical testing of the femur was performed at room temperature using a materials testing machine (MZ500D; Maruto, Tokyo, Japan). For stabilization, the mid-diaphysis of the femur was placed on two supports of the test apparatus that were 4 mm apart. The load of a three-point bending test was applied in the anteroposterior direction midway between the two supports. The breaking force was calculated using software that measures bone strength (CTR win, Ver 1.05). After breaking force measurement, the femurs were dried at 120°C for 6 h in a muffle furnace and the dry weights were recorded. The femurs were then incinerated at 800°C for 16 h and the ash was weighed. One hundred milligrams of bone ash was then dissolved in 2 mL of 37% HCl and diluted with distilled water. Calcium and phosphorus contents were determined by atomic absorption spectrophotometry (PerkinElmer; A Analyst 100 Spectrophotometer, Boston, USA).

Statistical analysis

Mean and standard deviation of all measurement values were computed by Microsoft Excel. The significance of differences in the groups was evaluated by ANOVA using SAS (Statistical Analysis System) and were considered significant for values of $P<0.05$. Post hoc analysis was carried out using Duncan's multiple-range test.

Results

Body weight gain and feed/water intakes

Up to 2 days after the operation, a decrease in body weight was noted in both the OVX and OVX+SAC groups due to post-operative stress but there was consistent increase in body weight and food intake afterwards (data not shown). The increase in body weight after ovariectomy was slightly higher in the ovariectomy group (0.98 g/day) compared to the sham group (0.84 g/day) (Table 2). Notably, SAC treatment further increased the body weight gain, although there was no statistical significance. In regards to feed intake, significant changes ($P<0.05$) in daily feed intake was seen in the OVX group (20.53 g/day) compared to sham group (19.15 g/day). In addition, SAC further enhanced the feed intake, leading to an increased feed efficiency ratio (FER). It is considered

Table 2. Body weight gain, feed intake and feed efficiency ratio (FER)

Treatment	Body weight gain (g/day)	Feed intake (g/day)	FER
Sham	0.84±3.76	19.15±0.98[a]	0.04±3.8
OVX	0.98±6.06	20.53±0.81b[b]	0.04±7.48
OVX+SAC	2.38±6.74	21.59±1.39[b]	0.11±4.84

Means with different superscript letters are significantly different at $P<0.05$. Sham; sham operation, OVX; no-treatment after ovariectomy, OVX+SAC; Sigma Anti-bonding Molecule Calcium Carbonate treatment after ovariectomy.

that such a difference in feed intake following SAC treatment had an influence on the change in body weight. On the other hand, there were no differences in water intake among groups (data not shown).

Organ weights

The absolute organ weights are shown in Table 3. Weights of the reproductive organs (uterus and vagina) were decreased significantly by ovariectomy, which were not recovered by SAC. Other organs including femurs decreased slightly after ovariectomy but there were no statistically significant changes following SAC treatment. It was found that SAC did not have a big influence on organ weights.

Hematology and blood biochemistry

In hematological analyses,, a statistically significant increase in eosinophils in the SAC treated group after ovariectomy was noted, although there was a big deviation (Table 4).

There were no significant changes in the blood parameters related to the hepatotoxicity (AST, ALT and ALP), hepatic

Table 3. The absolute organ weights of ovariectomized rats treated with SAC for 12 weeks

Organs	Sham	OVX	OVX+SAC
Body weight (g)	310.84±11.14	353.75±10.18	371.36±9.97
Uterus (g)	0.189±0.050[a]	0.038±0.011[b]	0.044±0.020[b]
Vagina (g)	0.113±0.0303[a]	0.050±0.021[b]	0.058±0.020[b]
Liver (g)	2.34±0.15	2.17±0.10	2.18±0.15
Kidneys			
Right (g)	0.324±0.021	0.268±0.003	0.263±0.021
Left (g)	0.329±0.033	0.271±0.012	0.269±0.011
Spleen (g)	0.184±0.022	0.169±0.020	0.186±0.021
Adrenal glands			
Right (g)	0.0127±0.0024	0.0093±0.0012	0.0094±0.0014
Left (g)	0.0128±0.0020	0.0092±0.0008	0.0094±0.0014
Brain (g)	0.656±0.031	0.604±0.031	0.553±0.022
Pituitary (g)	0.0055±0.0001	0.0038±0.0002	0.0042±0.0010
Femurs			
Right (g)	0.330±0.021	0.296±0.023	0.290±0.021
Left (g)	0.352±0.030	0.293±0.012	0.294±0.021

Sham; sham operation, OVX; no-treatment after ovariectomy, OVX+SAC; Sigma Anti-bonding Molecule Calcium Carbonate.

Table 4. Hematological values of ovariectomized rat treated with SAC for 12 weeks

Parameters	Sham	OVX	OVX+SAC
White blood cells ($10^3/\mu L$)	4.95±3.15	4.69±1.79	4.84±1.17
Neutrophils (%)	22.28±4.48	23.4±7.18	21.21±6.19
Eosinophils (%)	1.00±0.52[a]	1.16±0.27[a]	5.04±3.88[b]
Basophils (%)	0.04±0.05	0.04±0.05	0.02±0.04
Lymphocytes (%)	73.72±3.97	72.54±6.96	70.36±8.78
Monocytes (%)	2.68±0.78	2.58±0.33	2.50±1.44
Hematocrit (%)	44.16±2.47	45.30±1.23	43.94±2.18
Red blood cells ($10^3/\mu L$)	7.95±0.64	8.26±0.24	8.23±0.55
MCV (fL)	55.68±2.11	54.82±0.82	53.44±1.24
MCH (pg)	32.26±1.36	30.94±0.91	30.50±0.75
MCHC (g/dL)	57.94±1.88	56.40±1.33	57.14±0.76
Platelets ($10^3/mm^3$)	1,440.2±153.8	1,234.8±82.4	1,297.0±91.3

MCV; mean corpuscular volume, MCH; mean corpuscular hemoglobin, MCHC; mean corpuscular hemoglobin concentration, Sham; sham operation, OVX; no-treatment after ovariectomy, OVX+SAC; Sigma Anti-bonding Molecule Calcium Carbonate treatment after ovariectomy.

Table 5. Blood biochemical values of ovariectomized rats treated with SAC for 12 weeks

Parameters	Sham	OVX	OVX+SAC
AST (IU/L)	159.8±37.8	163.0±20.3	148.0±30.4
ALT (IU/L)	19.2±4.5	18.6±4.3	22.4±4.0
ALP (IU/L)	108.6±29.7	112.6±17.9	108.2±20.7
Glucose (mg/dL)	109.8±7.4	115.0±17.7	112.4±7.7
Triglycerides (mg/dL)	11.8± 6.1	19.8±6.6	20.6±7.7
Total cholesterol (mg/dL)	59.6±4.8	60.2±24.3	73.0±17.3
HDL (mg/dL)	20.8±2.5	19.8±6.8	23.8±5.2
LDL (mg/dL)	10.2±4.1	8.9±5.3	8.0±5.2
Total proteins (mg/dL)	5.0±0.2	4.7±0.2	4.7±0.2
Albumin (mg/dL)	3.0±0.1	2.7±0.1	2.7±0.1
BUN (mg/dL)	9.7±2.3	10.2±2.4	9.2±2.4
Calcium (mg/dL)	6.8±0.3	6.7±0.2	6.7±0.4
Phosphorus (mg/dL)	4.4±0.4	4.3±0.3	4.1±0.6

AST; aspartate transaminase, ALT; alanine transaminase, ALP; alkaline phosphatase, HDL; high-density lipoproteins, LDL; low-density lipoproteins, BUN; blood urea nitrogen. Sham; sham operation, OVX; no-treatment after ovariectomy, OVX+SAC; Sigma Anti-bonding Molecule Calcium Carbonate after ovariectomy.

Table 6. 17β-Estradiol concentration and bone metabolism markers of ovariectomized rats treated with SAC for 12 weeks

Treatment	17β-Estradiol (pg/mL)	Osteocalcin (ng/mL)	CTx (ng/mL)
Sham	4.536±0.343^{a}	3.398±0.294^{a}	4.751±0.244^{ab}
OVX	3.920±0.277^{b}	4.047±0.501b^{b}	5.082±0.462^{b}
OVX+SAC	4.217±0.247^{ab}	3.558±0.154^{a}	4.360±0.495^{ac}

Means with different superscript letters in the same column are significantly different at P<0.05 by Duncan's multiple-range test. Sham; sham operation, OVX; no-treatment after ovariectomy, OVX+SAC; Sigma Anti-bonding Molecule Calcium Carbonate treatment after ovariectomy, CTx; C-terminal telopeptides.

Table 7. Breaking force and values of ash, Ca and P of ovariectomized rats treated with SAC for 12 weeks

Treatment	Breaking force (kg)	BMD (mg/cm^2)	Ash (mg/dL)	Ca (mg/dL)	P (mg/dL)
Sham	7.113±0.035^{a}	0.2276±0.011^{a}	428.2±17.34	140.8±2.48^{a}	138.0±3.16^{a}
OVX	7.002±0.106^{b}	0.1965±0.012^{b}	409.5±8.39	136.0±3.10^{b}	130.3±7.72^{b}
OVX+SAC	7.1220±0.064^{a}	0.2276±0.012^{a}	419.8±8.76	147.5±5.33^{c}	138.1±5.39^{a}

Means with different superscript letters in the same column are significantly different at P<0.05. Sham; sham operation, OVX; no-treatment after ovariectomy, OVX+SAC; Sigma Anti-bonding Molecule Calcium Carbonate treatment after ovariectomy, BMD; bone mineral density.

energy storage and pancreatic injury (glucose), hepatic lipid metabolism (triglcerides, total cholesterol, DHL and LDL), hepatic protein synthesis (total proteins and albumin), renal injury and electrolyte balance (BUN, calcium and phosphorus) (Table 5). Especially, however, ALP, a bone growth marker, increased moderately in SAC-treated animals.

Blood markers of bone metabolism

Blood 17β-estradiol concentration significantly decreased following ovariectomy, and the decrease in 17β-estradiol due to ovariectomy was significantly restored by SAC treatment (Table 6). Such a result indicates that the decrease in estrogen concentration caused the acceleration of osteoclast generation, accelerating bone resorption, which might be prevented by SAC. Increases in osteocalcin and CTx were found in the ovariectomy group compared to sham group. But such increases in the bone metabolic factors were recovered to their normal levels following treatment with SAC.

Breaking force and bone mineralization of femurs

The breaking force of femurs of ovariectomized significantly decreased, indicative of the weakened bone integrity (Table 7). In parallel with the alteration of breaking force, BMD as well as the concentrations of ash, calcium and phosphorus also reduced by ovariectomy. Interestingly, however, the breaking force and related ingredients (BMD, calcium and phosphorus) were fully recovered by SAC treatment.

Discussion

Osteoporosis has been studied by many health professionals and nutritionists in order to prevent the disease. Two major methods are used to diagnose osteoporosis including radiology and biochemistry. Compared to radiology which is a static

analysis of bone condition, biochemical diagnosis is able to analyze the dynamic state of the bone by comparing the mutual relationship between markers of bone [22].

When the weight changes of ovary-removed rats were investigated, the OVX group showed significantly high weight gain compared to the control (sham) group [9,23,24]. Although there was a weight decrease during the first 1-2 days post-surgery caused by stress, weight increased over the 12-week period [25,26]. Similar to these results, when we removed both ovaries of the rats, body weights significantly increased in the experimental group. On the other hand, the food and water intake of the control group slightly increased, but was not significantly different. Such results show similarity with previous studies [25,26].

It has been attempted to investigate the form of osseous tissue and the kinetics of calcium metabolism by using calcium isotopes. However, because of the complexity of tests and the clinical limitation, a biological marker which has high sensitivity and applicability is needed to measure the bone turnover in osteoporosis. In addition, to evaluate the formation and absorption of the bone matrix, measuring the activity of alkali or acidic oxidase of osteoblasts and osteoclasts can be performed. Also, there is a way of measuring bone matrix components circulating in the system which are released during bone formation and absorption processes.

ALP increased in the serum depending on osteoblastic activity. ALP in the serum indicates positive correlation with other biological markers but has a negative correlation with bone density and it reflects increased bone turnover by bone loss after ovariectomy. However, because the rate of bone turnover can be increased by other diseases such as hyperthyroidism, a true correlation between ALP and osteoporosis may be skewed.

According to Delmas [27], ALP can be a possible marker for bone turnover but not suitable for bone loss because of the production of the enzyme is not only limited to the osseous tissue; the liver produces an isoenzyme of ALP which reduces the sensitivity and the specificity of the test. In our study, the concentration of the enzyme in serum increased, but did not show relevance with osteoporosis.

Known as a compound closely related with bone metabolism, 17β-estradiol is mainly produced by the ovary, corpus luteum, placenta, adrenal glands and testis. Remarkable fluctuations occur during the menstrual cycle and pregnancy in women. Dr. Fuller Albright first mentioned that sex hormone deficiency can cause osteoporosis. Wroolie *et al* [28] and Durador *et al* [29] reported ovariectomy-induced osteoporosis and menopause induced osteoporosis with significant reductions of 17β-estradiol in the blood. Also in this study, compared to the control group, the experimental group showed significant 17β-estradiol reductions which is consistent to previous studies [28,29]. Bone turnover rate is increased by reductions in estrogen, and bone loss rapidly progresses by increased bone absorption compared to bone formation.

Osteocalcin exists in bone and dentin and accounts for about 20% of non-collagen proteins in bone. It was reported that osteocalcin limits mineralization in *in vitro* experiments and bone density in mice without the osteocalcin gene is increased [18]. The concentration of osteocalcin is increased due to the rapid bone turnover rate after menopause but is decreased by injection of estrogen. Therefore, the increase of osteocalcin will likely increase bone turnover rate [27]. In this experiment after ovariectomy, the concentration of osteocalcin increased, and after the SAC treatment, the concentration remarkably decreased, indicating the changes of bone turnover rate; this suggests the the possibility of SAC as a treatment for osteoporosis.

According to a 2 year period follow-up study among the women who had femoral fractures, the baseline of CTx was increased compared to women without femoral fracture [13,16,30]. Same as in this study, CTx remarkably increased after ovariectomy, followed by a significant decrease with SAC treatment.

Low bone density is a risk factor and tends to progress into decreases in femoral bone density after menopause [9,31]. In this study, bone density of the OVX group showed low values compared to the sham group and SAC treatment increased BMD after ovariectomy.

The primary change after estrogen deficiency is an increase in the outflow of calcium from the skeleton. Due to the rapid bone loss, the secretion of parathyroid hormone decreases, causing a decrease in the calcium absorption in the intestine and finally resulting in the loss of calcium from the body and bone [2,32]. It was recently reported that over 2 years administration of calcium was more effective in decreasing bone loss of the placebo group in the study of effect of calcium supplement on bone density and fracture in women after menopause with 15 meta-analysis [17,18].

In this experiment, compared to the sham group, OVX had a significant decrease in calcium while the OVX+SAC group was not significantly different to the sham group, demonstrating decreased progression to osteoporosis. In summary, osteoporosis induced by ovariectomy could be alleviated by SAC treatment.

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CBHI and UAMS
(Winthrop P. Rockefeller Cancer Institute)

Project Title:
Study of the Role of Sigma Anti-bonding Calcium (SAC)
in Multiple Myeloma and Erythropoietin-Induced Osteoporosis

Pre-clinical Study

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BACKGROUND AND SIGNIFICANCE

Multiple myeloma (MM) is the second most common hematological malignancy. It mostly occurs at older ages and remains an incurable disease. MM is a B-cell cancer mainly characterized by the proliferation of malignant plasma cells in the bone marrow, the presence of monoclonal serum immunoglobulin, and the occurrence of osteolytic lesions. MM is closely associated with bone destruction. Osteoclasts (OCs) were shown to enhance bone destruction and support myeloma progression while osteoblasts (OBs) were shown to enhance bone formation and inhibit myeloma growth. In MM the activities of OCs were up-regulated while 0B activity was down-regulated. This evidence suggested that manipulating the activity of OCs and/or OBs can be a good candidate for a novel myeloma therapy.

C57BL6/KaLwRij mice spontaneously developed MM at 2+ years old with a frequency of about 0.5%. In the bone marrow of these mice, typical myeloma cells were found in the morphologic and immunohistochemical examination. On radiological images of the skeletons, osteoporosis with

occasional osteolytic lesions were found, mainly in the ribs, femur, and tibia. However, transplantation of mouse MM cells (5T33) into this mouse caused death by MM in 6-8 weeks. This model has been reported to recapitulate many features of human multiple myeloma [2].

The 5T33 cell line was established from bone marrow cells of MM-bearing C57BL6/KaLwRij mice at post-transplantation of 5T.

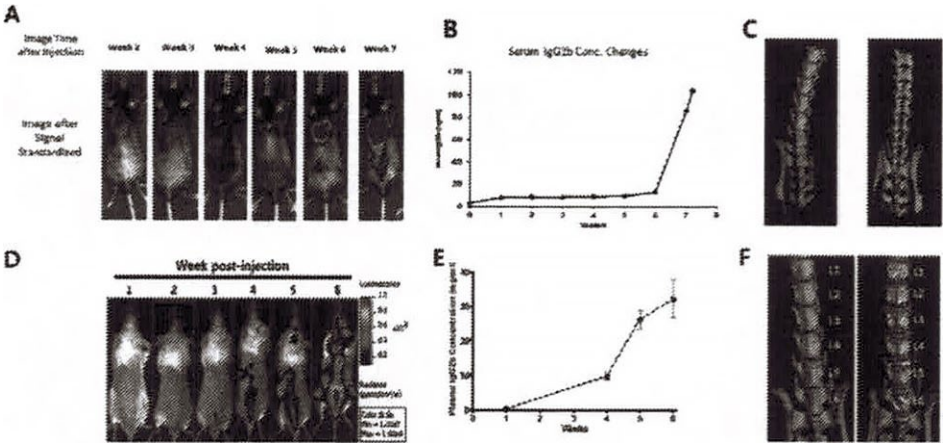
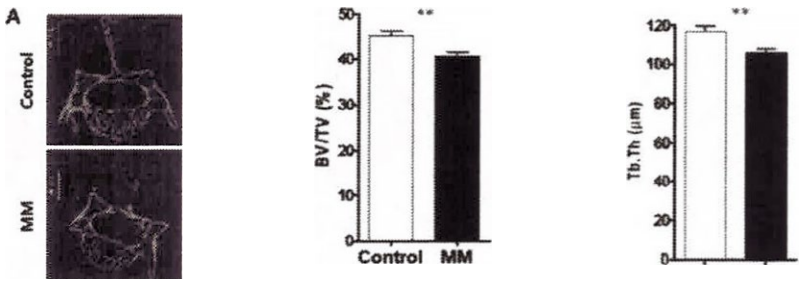


Figure 1. Multiple Myeloma Mouse Models 5TGM1-Luc Transplanted 57BL6/KaLwRij (A, B, C) and NSC (D, E, F).

(A, D) Weekly Bioluminescence Images from 5TGM1-Luc transplanted mouse (B, E) serum immunoglobulin levels C, F) 3D reconstructed images of spines from PCT scanning cells and the first of growth factor (IL-6) independent murine myeloma cell line. This cell line has provided valuable information on growth factor requirements, therapeutic susceptibility and genetic alterations inherent in myeloma development with the potential for direct application to human MM due to similar phenotypes [3]. The cell line 5TGM1 was subsequently established/subcloned from the 5T33 cell-transplanted MM bearing C57BL6/KaLwRij mouse [4]. The 5TGM1 in the transplanted C57BL/KaLwRij mouse caused osteolytic bone lesions with human myeloma bone disease characteristics. The 5TGM1 transplant C57BL/KaLwRij mouse model reproduced most of the major aspects of myeloma bone disease occurring in humans.



Histomorphometric Analysis of the 5TGM1-NSG Mouse Model. The spine was extracted from the terminated mouse due to the severity of MM symptoms, then scanned by PCT. (A) Representative cross-section image of 5th lumbar spine. Morphometric analysis of MM against vehicle control; (B) Percentage of bone volume/total volume; (C) Trabecular bone thickness Mean ± SEM, **: $p<0.01$. Includes osteolysis, hypercalcemia, significant loss of the trabecular bone density, and increased osteoclastic bone resorption associated with monoclonal gammopathy [4]. In addition, we recently transplanted the 5TGM1-Luc cells into the NOD-scid Il-2Ry^{null} (NSG) mouse and found significant bone lesions on its spine (Fig. 1 D, E, F). The spine from this model has been histomorphometrically analyzed and revealed that the 5TGM1-Luc cell transplanted NSG (5TGM1-NSG) demonstrated severe MM induced bone disease (unpublished data). These animal models with 5TGM1-Luc cells have served as a useful model not only to study myeloma tumorigenesis, but also to determine the efficacy of potential therapeutic agents.

Furthermore, we have human erythropoietin receptors (EPORs; wild type and mutant) knockin mouse model. The mutant receptor was cloned from a patient who has congenital and inherited polycythemia [5][6]. The human mutant type EPOR knock-in mouse has gain-of-function erythropoietin (EPO) signaling as it is shown in the polycythemia patient [5][7] while the human wild type-EPOR knock-in mouse has significantly low EPO signaling [8]. Lately, EPO has demonstrated both bone formation and resorption [9-13]. On this mutant EPOR expressing mouse we found severe osteoporosis after old age (more than 1 year) (unpublished data). This model has not been thoroughly investigated for bone pathology, but clearly suggests that older polycythemia patients who inherit the gain-of-function EPOR mutation may be predisposed for osteoporosis.

According to company website (https://markets.businessinsider.com/news/stocks/cbhipresents-innovative-siama-anti-bondina-calcium-sac-in-medical-industry-1027379504), Sigma Anti-bonding Calcium (SAC) is highly soluble in water and increases bone mineral density up to 80%. SAC is the first ionized calcium that can be delivered into the body and deposited into the bone. In myeloma patients, during the first bortezomib treatment cycle, serial serum

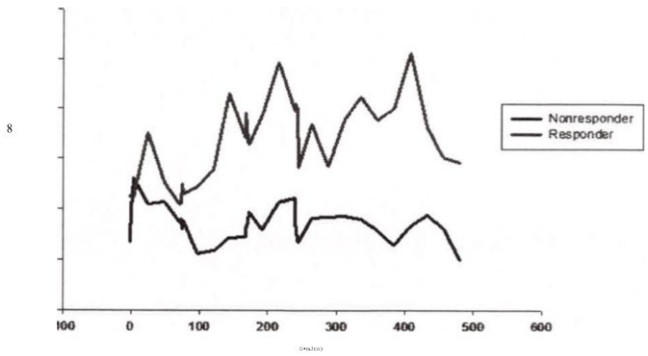


Figure 2. Averaged PTH level changes during the first cycle of bortezomib

Parathyroid hormone (PTH) measurements revealed a significant difference in responders from the non-responder group against bortezomib [1]. However the non-responder group showed PTH release when Panobinostat with bortezomib was injected as shown in Figure 2. Extracellular calcium Figure3. Averaged PTH level changes during the first cycle of bortezomib. The averages of individual serum PTH levels from 16 patients are plotted here; 7 responders and 9 non-responders. Figure was adapted from manuscript [1].

Levels regulate PTH release, thereby controlling serum calcium levels systematically. PTH may induce bone resorption via osteoclasts. Our recent report demonstrated that PTH axis plays important roles in anti-MM activity [14].

We recently found the thymus-derived PTH (TPTH) and parathyroidectomized (PTX) mice do not develop MM after 5TGM1 injection. After PTX, the mouse recovered the serum calcium levels immediately [15] [16]. We strongly believe that PTH plays an important role in anti-myeloma activity via uncovered mechanisms. These uncovered mechanisms may include ionized calcium that can be introduced by SAC.

HYPOTHESIS

We postulate that SAC stimulates bone formation preventing myeloma cell growth and aging-related osteoporosis, and/or healing myeloma induced bone lesions.

SPECIFIC AIMS

To investigate the effect(s) of SAC on multiple myeloma and EPO-induced osteoporosis, we propose the following:

1. We will perform a pharmacokinetics study of SAC in the mouse through different routes.
   Through this, we will discover the achievable serum calcium concentration and maintainable serum concentration after SAC treatments through various routes.
   We will also test serum PTH and Vit. D levels to evaluate an impact on a normal calcium physiology in the body.

2. We will evaluate the biological safety of SAC in mice.
3. We will evaluate the in vivo effect(s) of SAC in the mouse-MM model and EPO-induced osteoporosis

EXPERIMENTAL APPROACH

Aim 1: We will perform the pharmacokinetics study (absorption and clearance) of SAC in the mouse through different routes.

Calcium is a vital component in the body and its concentration (8.4-11.4 mg/dL) is tightly regulated. Any variation outside this range can cause serious issues. Although the previous work provided SAC treated water at 0.0012% ad libitum[17], which is a good method for a long-term treatment, but it may increase individual variations.

We will test three different routes (i.e. intraperitoneal, intravenous, gavage) to administer SAC to the mice at the established dosage (5 and 10 mg/dL in a 25-30 g mouse, 1.5-1.8 ml total blood volume). Blood will be drawn a week before injection (baseline), and 30 min, 1, 3, 6, 12, 24, 48, 72, 96 hours, 7 days after injection. The collected blood will be analyzed for serum calcium to find achievable serum calcium concentrations. Since it is calcium that is tightly regulated once in the body, 25(OH)D and mouse PTH levels will be tested in the blood sample collected here. Once we select the optimal concentrations of SAC, we examine dosing interval (how many times a week) to maintain the selected concentration by serum calcium assay.

For long-term treatment (several months) we will test 0.0012 % and ?? % ad libitum as well. The blood will be drawn bi-weekly for 3-6 months. In this method achievable serum concentration, PTH and 25(OH)D will be tested in the blood.Expected findings from this Aim: We will find the achievable serum concentrations for each route after SAC treatment and evaluate the effect(s) of SAC on calcium homeostasis in mice. These results lead us to postulate the potential biological impact(s).

Aim 2: We will evaluate the biological safety of SAC in mice.
Although the previous study already looked at the hematological analysis, the study used the ad libitum and relatively low concentration methods. It would be better to re-evaluate these at higher serum SAC concentrations.

We plan to maintain a high SAC concentration for one month in the route selected in Aim 1.

Four groups of mice will be studied in this specific aim; having control and SAC treated groups with 3 different routes as follows;

A. PBS-treated Control Groups (each group has 6 mice, 3F+3M)

B. SAC-treated Group (each group has 10 mice, 5F+5M)

a) Intraperitoneal Injection

b) Intravenous Injection

c) Gavage

At the end of month, we will evaluate the CBC counts and body/organ weights of the SAC-treated mice compared to the PBS-treated mice (control).

In addition, we will evaluate bone formation activity by various imaging methods (i.e. bone densitometry (dual-energy X-ray absorptiometry; DEXA), magnetic resonance imaging (MRI), micro-computed tomography (CT), histomorphometric analysis, and serum bone metabolic markers).

Expected findings from this Aim: We will find possible SAC treatment conditions and their effects on the bones, particularly spines. Although the spine is a very critical organ, it is difficult to study due to its complex structure. In recent years, we have been studying the spine and developing multiple ways to evaluate it. With this Aim, we will evaluate the potential benefits/disadvantages to the skeleton through the various means of SAC treatment.

Aim 3: We will evaluate the in vivo effect(s) of SAC in the mouse-MM model and EPO-induced osteoporosis

As described in Background section, we have developed several animal models recapitulating human diseases; multiple myeloma bone disease (MMBD) and gain-of-function EPOR derived osteoporosis.

We will evaluate the beneficial effects to the skeletal system and myeloma progression in the mouse models of the SAC-treated mice.

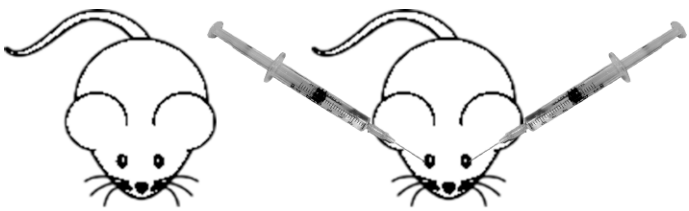
a. In the MMBD model, we will perform in vivo IVIS imaging (as shown in Figure 1 A and D) for myeloma cell growth and evaluate bone formation as described in Aim 2.

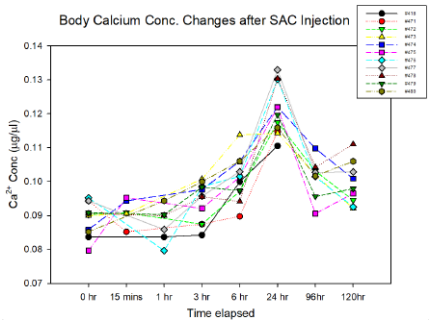
b. In gain-of-function EPOR derived osteoporosis, the gain-of-function EPOR derived osteoporosis may be developed as the mouse ages. We may start the SAC treatment from old age (6 and 9 months of age) until it is 12 months old using the ad libidium method. At 12 months of age, mice will be sacrificed and bone formation will be evaluated as described above.

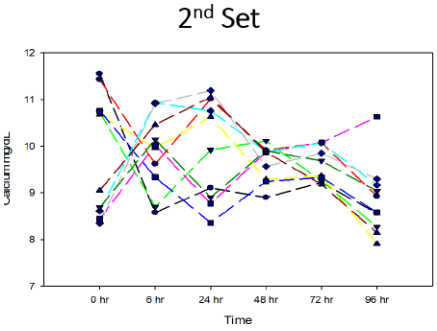
Expected finding from this Aim: We will find the effect(s) of SAC on two disease models: MMBD and osteoporosis. Compared to normal physiology, osteoporosis causes a mild imbalancing in bone metabolism, while cancer cells induce extremes. In each case, the application of SAC may require a different regimen. We may find various SAC effects or treatments in response to two distinct disease models

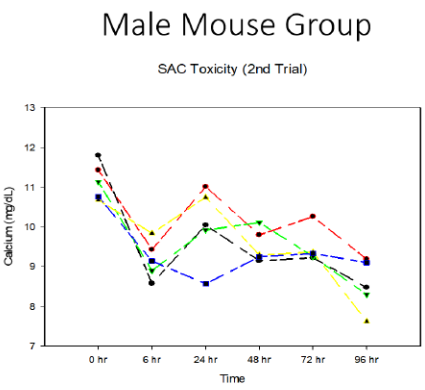
UAMS and CBHI
Interim Report on Oct 27. 2020

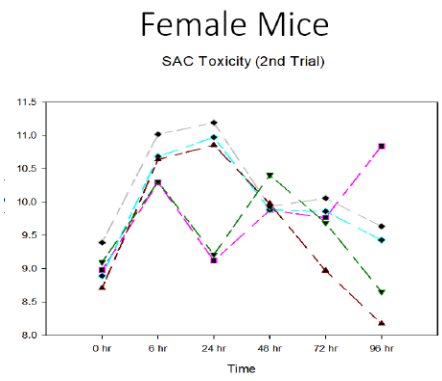
To find the kinetics of SAC in mouse,
10ug/dL (plain IV solution without dilution) has been injected. Based on an assumption that mouse contains 1.5~1.8ml of serum. 0.150~0.180mg per mouse is aimed to be administrated. To minimize local shock of SAC injection, the injection was divided into one (100ul) per eye, then another eye after ~10 min.

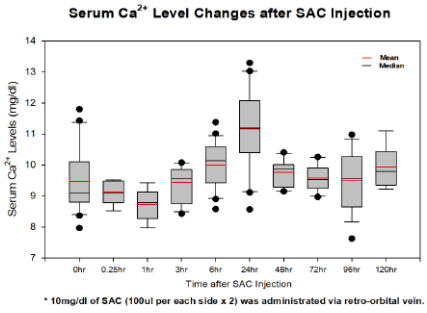



Body Calcium Conc. Changes after SAC Injection
Ca2+ Conc (μg/μl)
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0 hr
15 mins
1 hr
3 hr
6 hr
24 hr
96hr
120hr
Time elapsed


2nd Set
Calcium mg/dL
12
11
10
9
8
7
0 hr
6 hr
24 hr
48 hr
72 hr
96 hr
Time


Male Mouse Group
SAC Toxicity (2nd Trial)
Calcium (mg/dL)
13
12
11
10
9
8
7
0 hr
6 hr
24 hr
48 hr
72 hr
96 hr
Time


Female Mice
SAC Toxicity (2nd Trial)
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
0 hr
6 hr
24 hr
48 hr
72 hr
96 hr
Time


Serum Ca2+ Level Changes after SAC Injection
Serum Ca2+ Levels (mg/dl)
Mean
Median
14
13
12
11
10
9
8
7
0hr
0.25hr
1hr
3hr
6hr
24hr
48hr
72hr
96hr
120hr
Time after SAC Injection
* 10mg/dl of SAC (100ul per each side x 2) was administrated via retro-orbital vein.

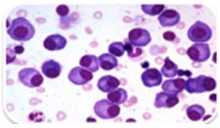


Evaluation of SAC Effects on Normal and Patho-Physiology

Donghoon Yoon, PhD

Associate Professor
Myeloma Center
Winthrop P. Rockefeller Cancer Institute
The University of Arkansas for Medical Sciences

9/29/2022



UAMS Myeloma Center

The UAMS Myeloma Center is the most comprehensive center in the world for research and clinical care related to multiple myeloma and related diseases. Our team of leading scientists and clinicians has pioneered many advances that have become standards of care, leading to improved survival rates. The Myeloma Center has over 30 years of excellence program and is known for its "bench-to-bedside" approach, continually translating advances in the laboratory into breakthrough clinical treatments. It has recognized the world leading institution for myeloma. More than 11,000 patients from every state in the United States and more than 50 foreign countries have come to the Myeloma Center for their care. We have performed more stem cell transplants than any other center in the world.

The UAMS Myeloma Center has unique score system (GEP70 score) to classify patients, predict outcome, and decide treatment. The GEP70 is scored based on patient gene expression profiles. Due to the cumulative GEP data from patients during last 30 years the Multiple Myeloma Database includes more than 4,000 GEP from thousands of MM patient samples who belongs to different risk groups.

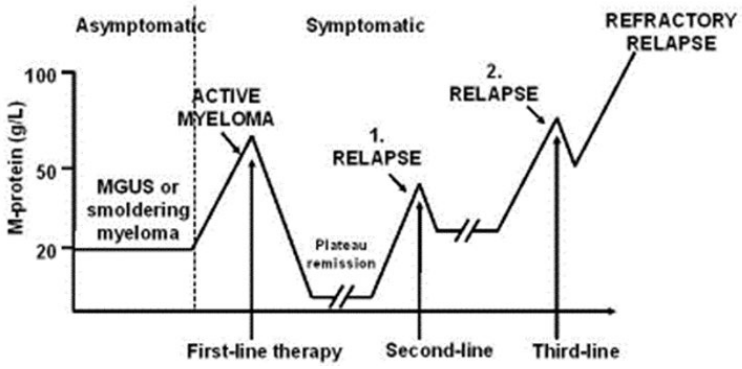
Multiple Myeloma

- MM is incurable B-cell malignancy characterised by abnormal proliferation of plasma cells able to produce a monoclonal immunoglobulin (M protein)
- Represents 10% of blood cancers, ~1% of all cancers and 2% of all cancer deaths
- 5 year overall survival rate fluctuates according to the risk (Low risk to high risk; 75-80% to 15%)
- The median age at diagnosis is 66 years. 10 percent of patients are younger than 50 years.
- Multiple myeloma is clinically characterized by:
  - Excessive numbers of abnormal plasma cells in the bone marrow
  - Overproduction of monoclonal immunoglobulin (free monoclonal κ and λ light chains)
  - MM-related events (hypercalcemia, Renal failure, Anemia, Bone lesions,; CRAB)



The Bone Lesions are found in 79% of patients with newly diagnosed MM, and in 84% of patients, skeletal lesions developed during the course of the disease (Kyle et al., 2003). They are highly associated with inferior survival, reduced quality of life, and increased healthcare costs (Zangari and Suva 2016). The bone lesions are occurred by increasing bone resorption of osteoclast cells and reducing the bone formation of osteoblast cells. Mineralization, the process of deposition of calcium, phosphate, and other ions in the bone matrix, is an essential process to generate bones.

Treatment Outcome



MM Bone Disease

By the International Myeloma Working Group (IMWG), it includes the presence of one or more osteolytic lesions on skeletal radiography, CT, or PET/CT as myeloma defining event.

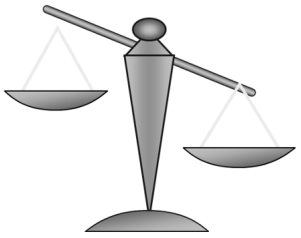
- MM bone lesions are found in approximately 70% of MM patients at diagnosis; skull, spine, rib cage, and pelvis are the most commonly involved disease sites.
- Bone lesions cause bone pain, pathologic fractures, spinal cord compression, and hypercalcemia.
- These features are highly associated with inferior survival, reduced quality of life, and increased healthcare costs.
- A bone lesion in MM results from the dysregulated bone remodeling caused by interactions between MM cells and the bone marrow microenvironment.

MM – Bone Disease

In patients with MM the process of bone remodeling (Synthesis-Resorption) and bone homeostasis is disrupted.

Tumor cells (either directly or through complex interactions with the bone marrow microenvironment):

- stimulate the bone resorptive activity of osteoclasts (IL-6, RANK-RANKL, etc)
- suppress the bone-forming activity of osteoblasts, ultimately causing significant bone destruction (pathological fracture).



Increased osteoclastic activity/ decrease bone formation

We Developed a New Animal Model with Severe MMBD

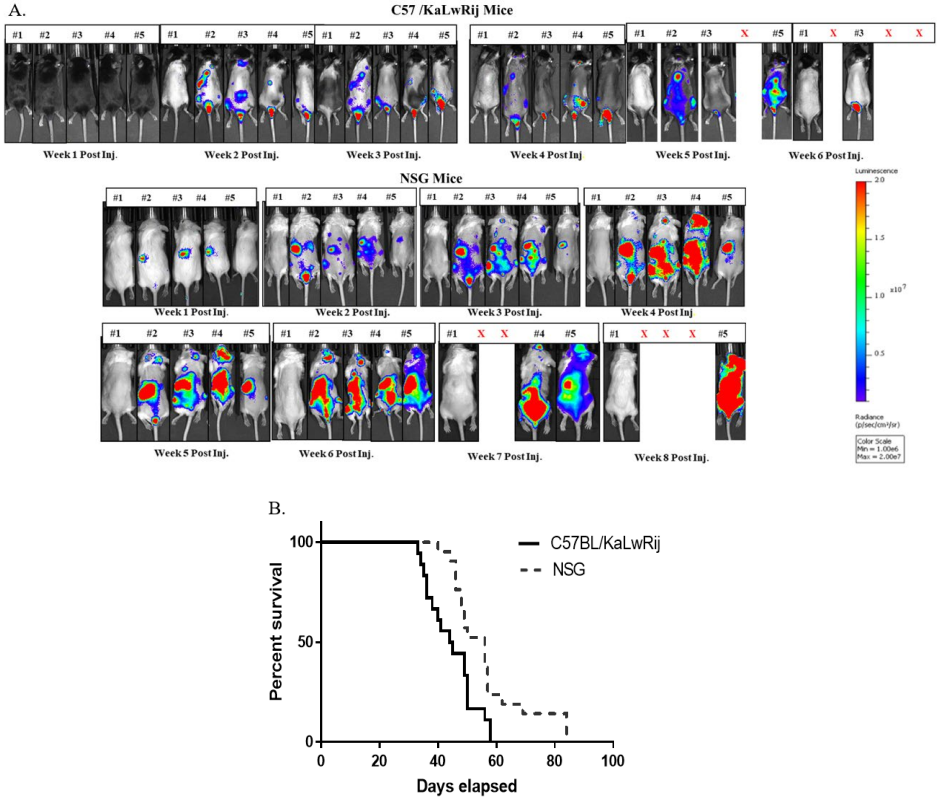


Figure 1 (A) Representative weekly BL images of 5TGM1-Luc transplanted C57BL/KaLwRij and NSG Models. All BL signals were normalized in one scale as shown. 1x106 5TGM1-Luc cells in 100 ul of PBS or PBS alone were injected to C57BL/KaLwRij or NSG mice (equal genders in 8~12 weeks old age) via the tail vein. In each group, the first one (#1) is a control whereas #2 ~ #5 are 5TGM1-Luc injected mice. (B) Mice were monitored weekly. When the mouse meets endpoint criteria, it is sacrificed and recorded. Survival curves representing 18 mice from C57BL/KaLwRij (solid line) and 21 Mice from NSG (dotted line). Median survival was 56 days for NSG compared to 44.5 days for C57BL/KaLwRij. The Mantel-Cox test showed P=0.0062.

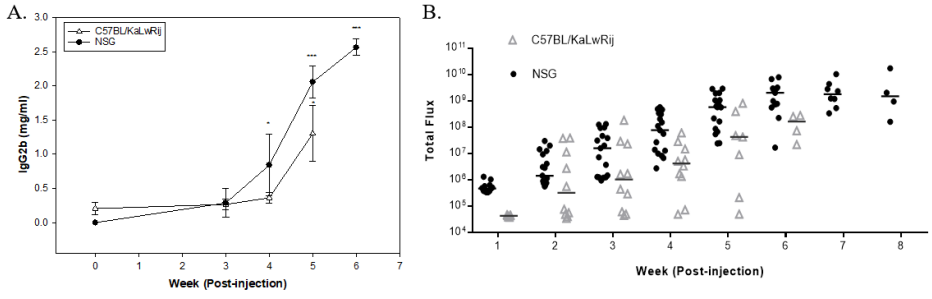
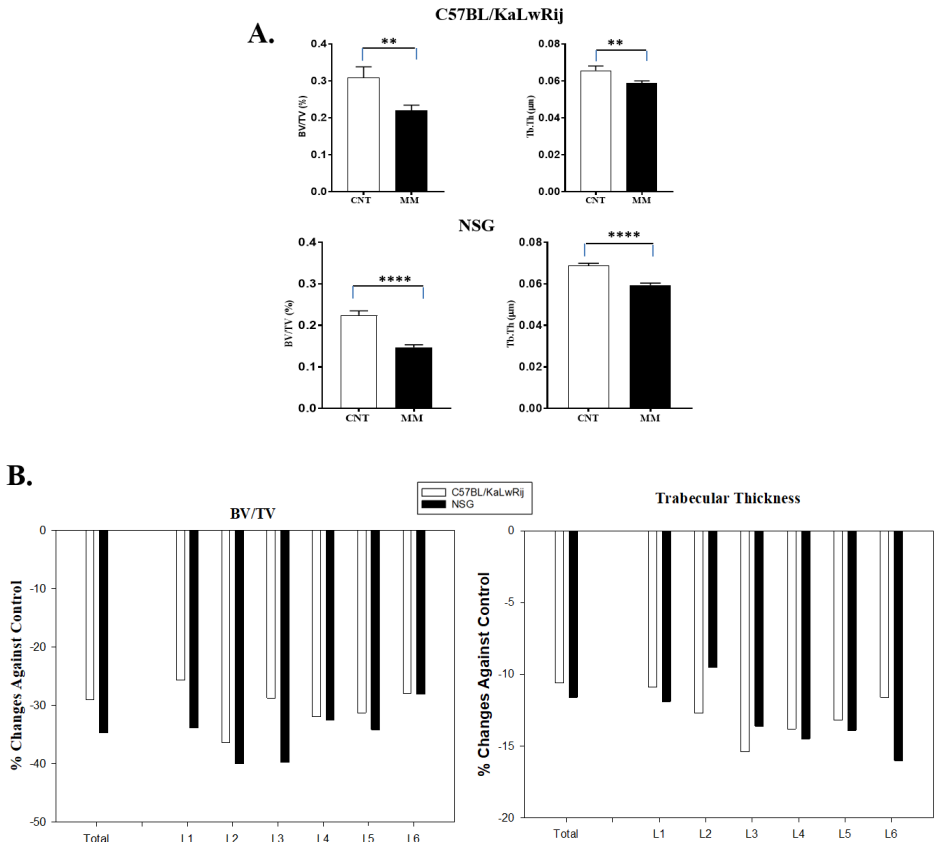
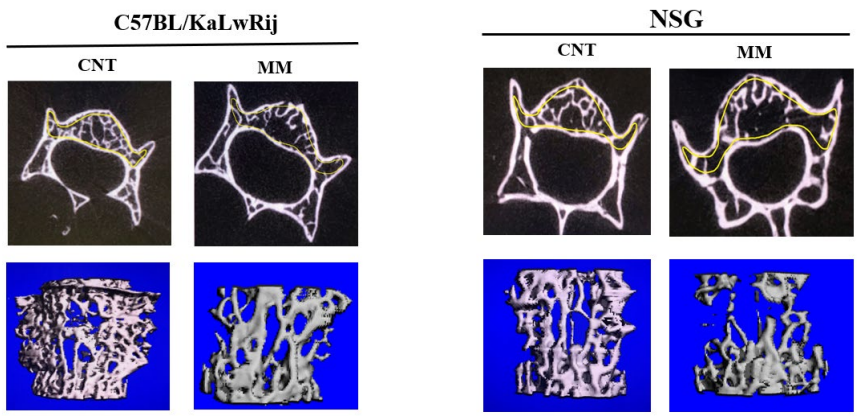


Figure 2 Monitoring myeloma progression using two different parameters; serum IgG2b levels and Whole-body bioluminescence signals. 1x106 5TGM1-Luc cells in 100 ul of PBS or PBS alone were

injected to C57BL/KaLwRij or NSG mice (equal genders in 8~12 weeks old age) via the tail vein. Every week BL imaging was taken and blood were drawn. (A) IgG2b were measured from plasma using ELISA kit. Mean serum IgG2b levels were calculated and expressed from NSG control mice and C57BL/KaLwRij. All undetectable IgG2b levels were brought to 0 mg/ml. Data are expressed as mean ± S.E. *P< 0.05, **P< 0.01, ***P< 0.001 compared with their own baseline. (B) From weekly image, total flux values were obtained from the regions of interest covering whole body of mouse. All total flux values (closed circle or open triangle) with median (line) are expressed as progression of tumor burden.

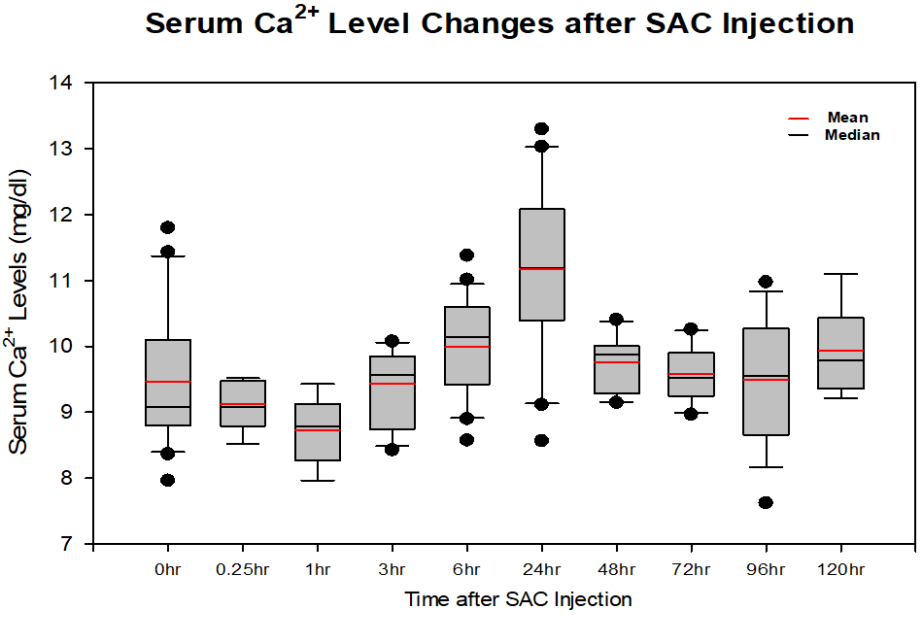


C.



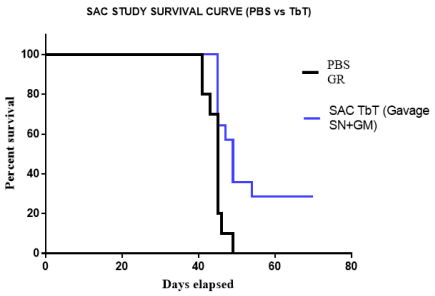
Conclusion

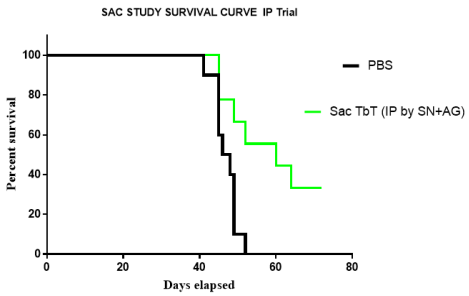
- Systemic injection of 5TGM1 into both models developed MMBD at axial skeleton areas.
- NSG mouse model uniformly progresses MM and develops severe MMBD than C57BL/KaLwRij model.



*** 10mg/dl of SAC (100ul per each side x 2) was administrated via retro-orbital vein. 0.02mg/mouse was administrated.**

Gavage versus Intraperitoneal (IP) injection



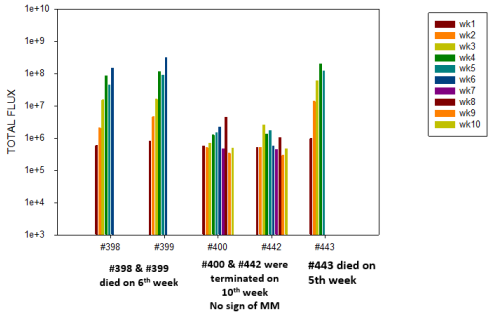


Mouse survived from MM progression.

Week-10

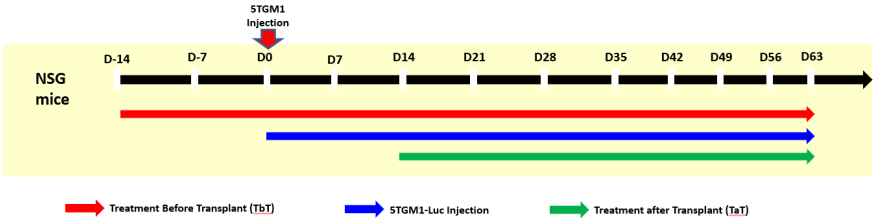


#95 TbT Group

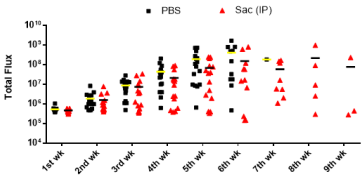


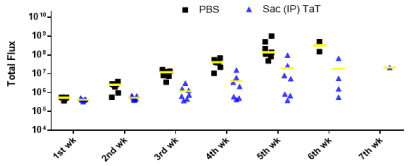
Can bone anabolic agent inhibit myeloma growth in our animal model?

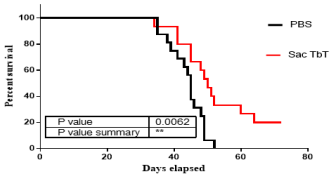
- **Animal**
  1. NSG mice (7 ~ 12 weeks old)
  2. 1e6 5TGM1-Luc cells were injected via tail vein
- **Group:**
  1. Control (PBS)
  2. Treatment started from 2 weeks before transplant.
  3. Treatment started from 2 weeks after transplant.
- **Weekly**
  1. Imaging for myeloma progression using Bioluminescent imaging (BLI)
  2. Body Weight check when mice were treated.
  3. Blood drawn



SAC IP treatment delays MM Progression in 5TGM1-engrafted NSG mouse.

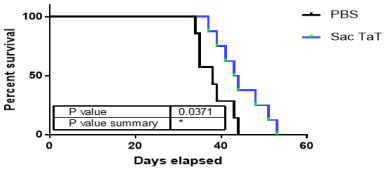






	PBS GR	Sac (IP)
Median survival	45	50

PBS GR	Sac (IP)
16.000	15.000



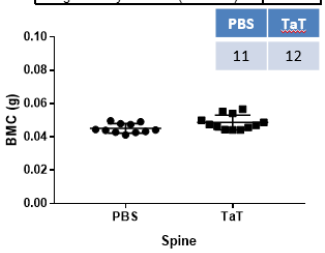
	PBS GR	TaT GROUP
Median survival	38	43.5

PBS GR	TaT GROUP
7.000	8.000

SAC IP treatment increase bone mineral densities (BMD) and contents (BMC) in 5TGM1-engrafted NSG mouse.

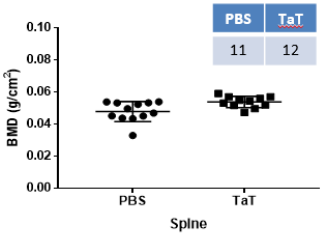
Unpaired t test	
P value	0.0377
P value summary	*
Significantly different (P < 0.05)?	Yes

PBS	TaT
11	12



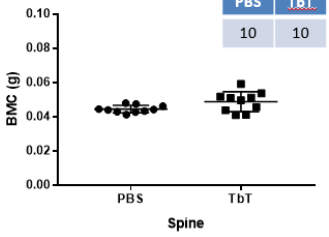
Unpaired t test	
P value	0.0106
P value summary	*
Significantly different (P < 0.05)?	Yes

PBS	TaT
11	12



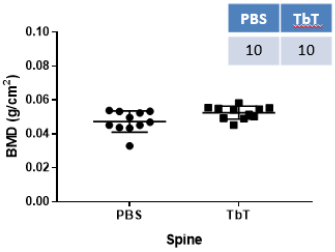
Unpaired t test	
P value	0.0420
P value summary	*
Significantly different (P < 0.05)?	Yes

PBS	TbT
10	10

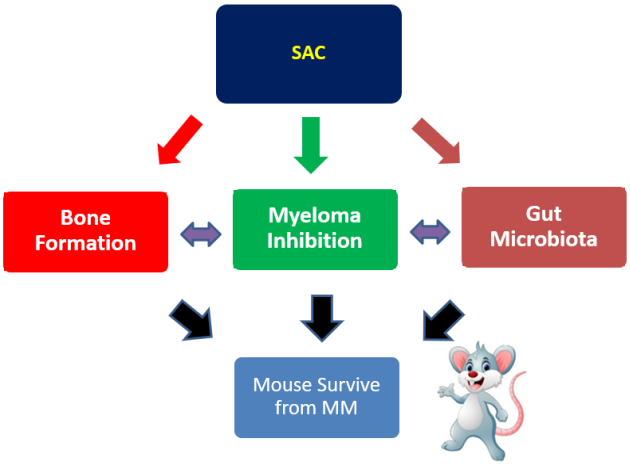


Unpaired t test	
P value	0.0266
P value summary	*
Significantly different (P < 0.05)?	Yes

PBS	TbT
10	10

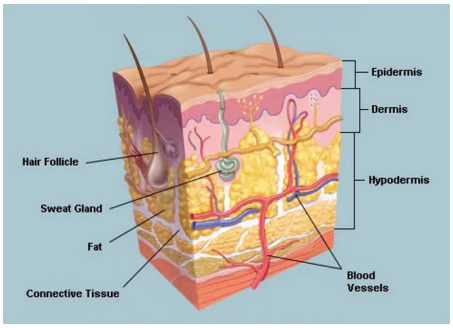


Proposed Model



SAC Effects on Skin

Structure of Human Skin



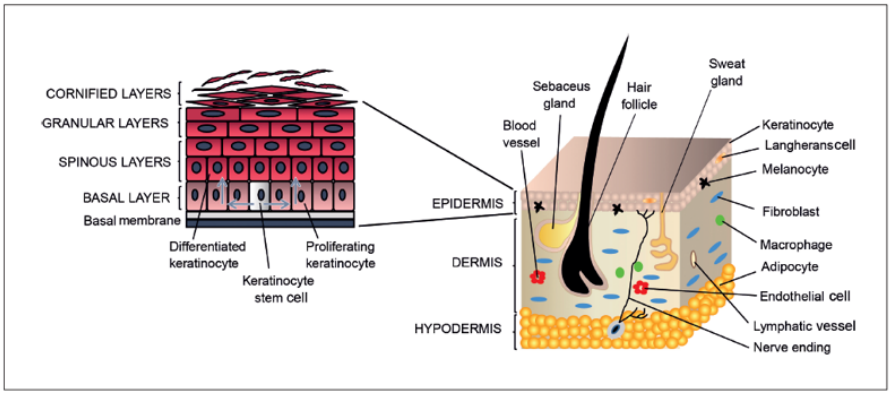
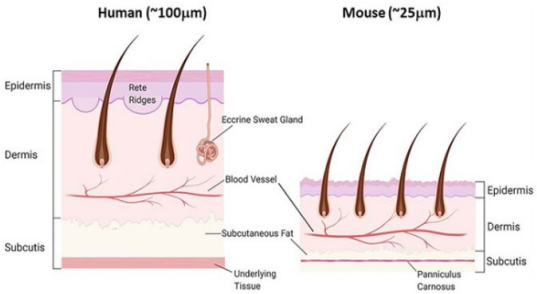


Fig. 1: Schematic human skin organization

Skin is structured into three tissues: the epidermis, the dermis, and the hypodermis. The epidermis is a stratified epithelium constituted of keratinocytes organized into four major layers (basal, spinous, granular, and cornified) at progressive differentiation stages. The epidermis also contains Langerhans cells of the innate immune system and melanocytes, which express melanin. The dermis is mainly populated by fibroblasts releasing the extracellular matrix, and hosts several immune cells, vascular and lymphatic vessels, and nerve endings. The hypodermis is mainly composed of adipocytes. Hair follicles, sebaceous and sweat glands constitute the skin appendages.

Comparisons of Human and Mouse Skin



Shi H, et al, "The contradictory role of androgens in cutaneous and major burn wound healing," 2021, Burns & Trauma, 9, tkaa046 DOI: 10.1093/burnst/tkaa046

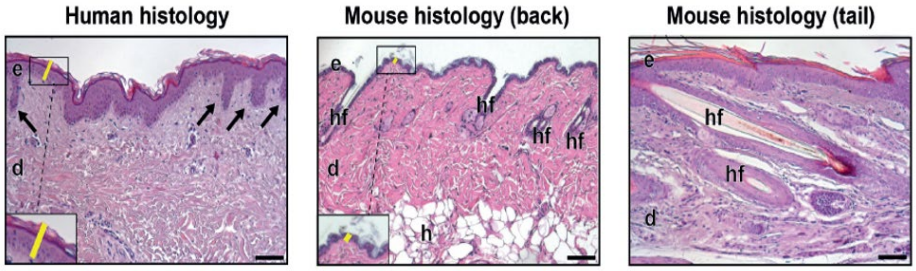
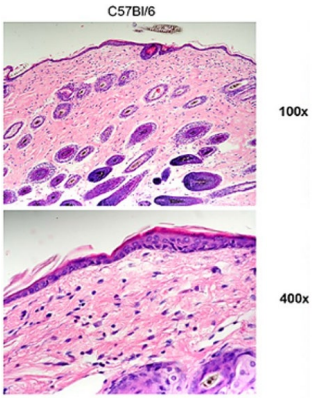


Fig. 2: Features of human and animal model skin

Upper panels: Histological comparison of human skin with mouse back and tail skin. Human skin is much thicker than mouse skin in the epidermal (yellow bars), dermal, and subcutaneous (fat layer) compartments. Moreover, human epidermis has projections (arrows) that protrude into the dermis, unlike mouse skin. In the mouse, hair follicles (hf) are found all over the body, whereas these are generally scarce in human skin. Fur has a protective function and mouse skin has less developed differentiated cell layers forming the epidermal barrier. Mouse tail skin, which has a lower number of hairs, has a thicker epidermis with increased cell layers compared to back skin and a more developed external stratum corneum. e, epidermis; d, dermis; hf, hair follicle. Bars: 50 μm.

Dellambra E, et al., "Non-animal models in Dermatological Research," 2019, ALTEX 36(2), 177-202.

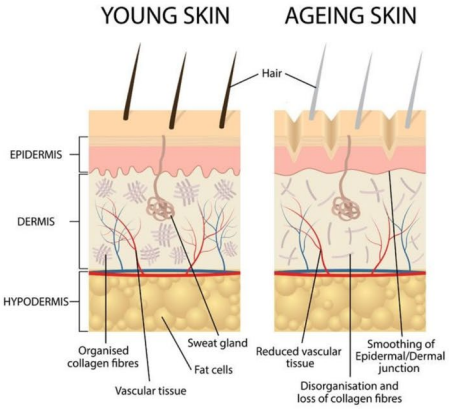
Normal C57BL/6 Mouse Skin



Knitlova, J. et al, "Histological features of the skin of Nc/Nga, Balb/c and C57Bl/6 mice 7 days after mock or WR inoculation by vaccination stamp." 2015, PLOS ONE.

Figure.
https://doi.org/10.1371/journal.pone.0114374.g002

What does aging mean at skin?



Aging skin tends to appear:
- Thinner and more transparent
- Slacker (saggier and looser)
- More fragile and more prone to damage (bruising) due to thinner blood vessel walls
- Drier
- Wrinkled
- Duller
- Rougher
- Unevenly toned. With age spots and possibly lesions.
- Different in shape

https://apothekari.com/aging-skin-7-things-that-can-happen/

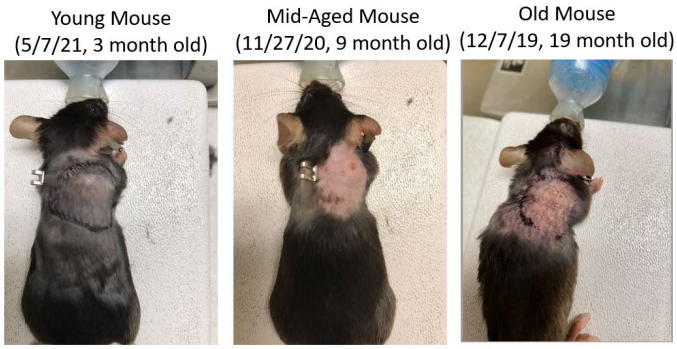
What changes in the skin during aging?

- Changes in the Epidermis. This outer barrier of our skin protects us from environmental insults, such as bacteria and ultraviolet (UV) radiation. Due to the natural aging process and sun exposure, changes occur. The most obvious one occurs at the epidermal/dermal junction – the space between the epidermis and the dermis – where a flattening of ridges occurs. This results in less surface contact of the epidermis and dermis

and leads to a reduced exchange of nutrients between these two parts. Aging skin doesn't get as much nourishment.

- Changes in the Dermis. The dermis sits under the epidermis and contains a very complex arrangement of proteins, including collagen and elastin, which are responsible for skin's strength and elasticity. Fibroblasts (skin cells) start producing fewer proteins:
- Elastin, which gives our skin elasticity and rebound. The result is sag.
- Collagen, which gives our skin firmness contributing to a significant reduction in elasticity. The result is thinner more fragile skin.
- Glycosaminoglycans (GAGs), which help to keep skin hydrated. The result is more dryness. And, there are fewer Blood Vessels, resulting in less blood volume and leaving the area deficient in nutrients.
- Changes in Fat Tissue. The subcutaneous fatty layer (also called the hypodermis) below the skin starts to shrinks, resulting in sagging. Fat is depleted and accumulates in areas including the forehead, the mouth, the eyes, the nose, the jaws and the cheeks. In young skin, fat tissue is widely distributed, but in aged skin fat tends to accumulate in pockets, which droop and sag due to the force of gravity.

Various aged female mice were treated with SAC Cream or Control Cream.



1. Animal; C57/BL6 mice in three different age groups (3, 9, 19 months old, Female)
2. Five mice per cage were maintained during the experiment. We randomly selected one mouse for Control cream within a cage.
3. Applied cream twice a day from the day for 4 weeks period.
4. Weekly shaved the dorsal region
5. Biopsy was taken at pre-, each week for 4 weeks



SAC Cream for Atopic eczema

By CHBI Canada

What is the filaggrin gene mutation atopic eczema?

In about 10% of our population in the world, this gene is faulty. This results in reduced production of normal filaggrin. And this gene is faulty in more than 50% of people with atopic dermatitis.

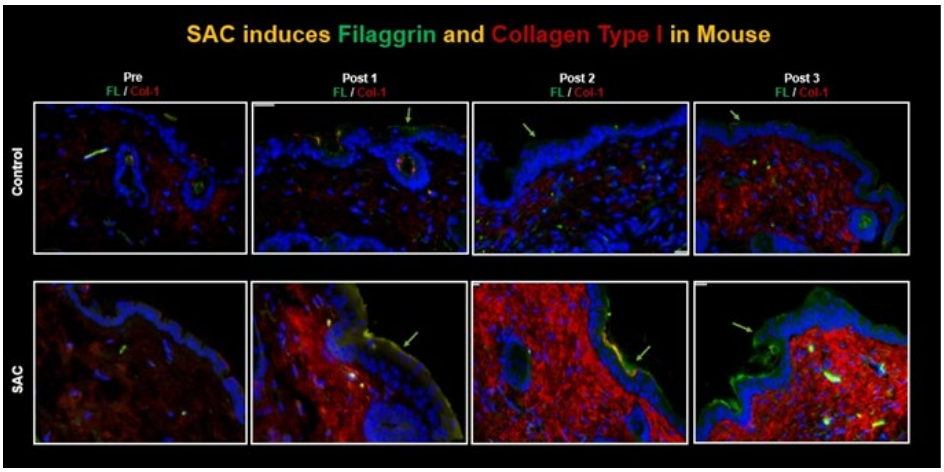
Mutations in the FLG gene are strongly associated with a skin disorder called atopic dermatitis (also known as atopic eczema). This condition is characterized by dry, itchy skin and red rashes.

The FLG gene provides instructions for making a large protein called profilaggrin, which is found in cells that make up the outermost layer of skin (the epidermis). Profilaggrin is cut (cleaved) to produce multiple copies of the filaggrin protein, which is important for the structure of the epidermis. The profilaggrin molecule can contain 10, 11, or 12 copies of the filaggrin protein, depending on the genetics of the individual. Further processing of the filaggrin protein produces other molecules that play a role in hydration of the skin.

The epidermis acts as a barrier to help minimize water loss and protect the body from foreign substances, including toxins, bacteria, and substances that can cause allergic reactions (allergens), such as pollen and dust mites. Filaggrin plays an important role in the skin's barrier function. It brings together structural proteins in the outermost skin cells to form tight bundles, flattening and strengthening the cells to create a strong barrier. In addition, processing of filaggrin proteins leads to production of molecules that are part of the skin's "natural moisturizing factor," which helps maintain hydration of the skin. These molecules also maintain the correct acidity (pH) of the skin, which is another important aspect of the barrier.

Through rat skin experiments, sac cream prepared using sac shows that the amount of filaggrin in the skin epidermis layer dramatically increases. This experimental result is evidence that SAC strongly prevents mutation when the filaggrin gene is replicated and increases the reduced amount of filaggrin. In addition, the amount of collagen in the skin layer increased rapidly, making the skin thicker and much healthier than the

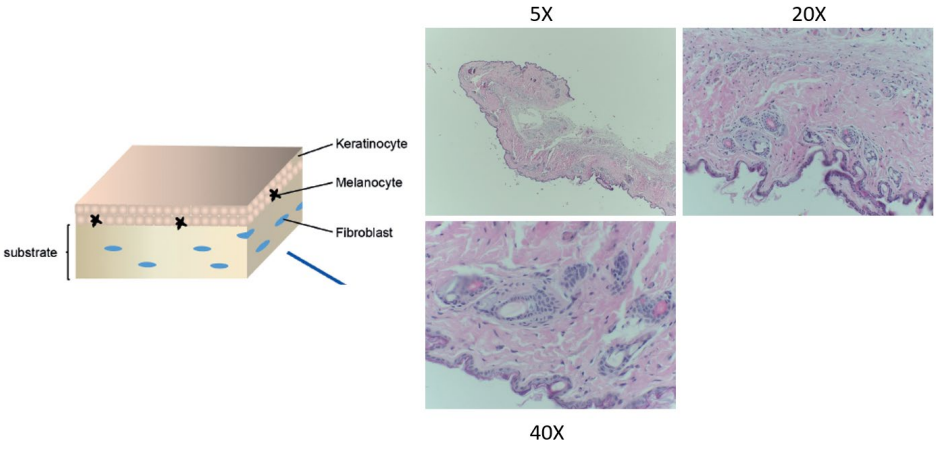
control group.

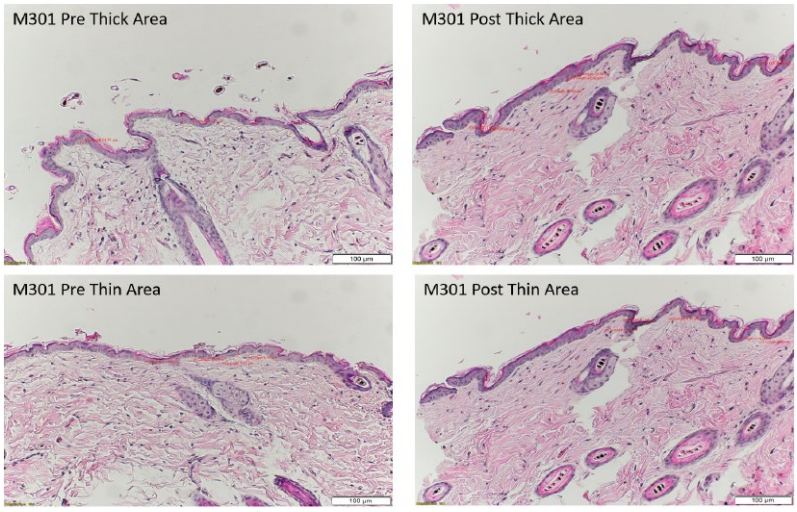


Provided by UAMS

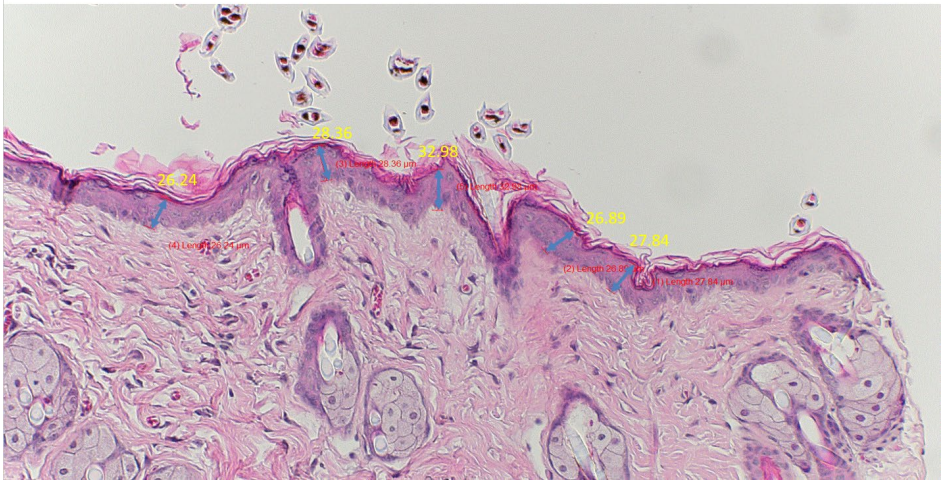
SAC Cream for Atopic eczema is expected to bring news of hope to many people suffering from atopic eczema skin.

A punch biopsy on the mouse dorsal skin allows to examine epidermis and dermis.

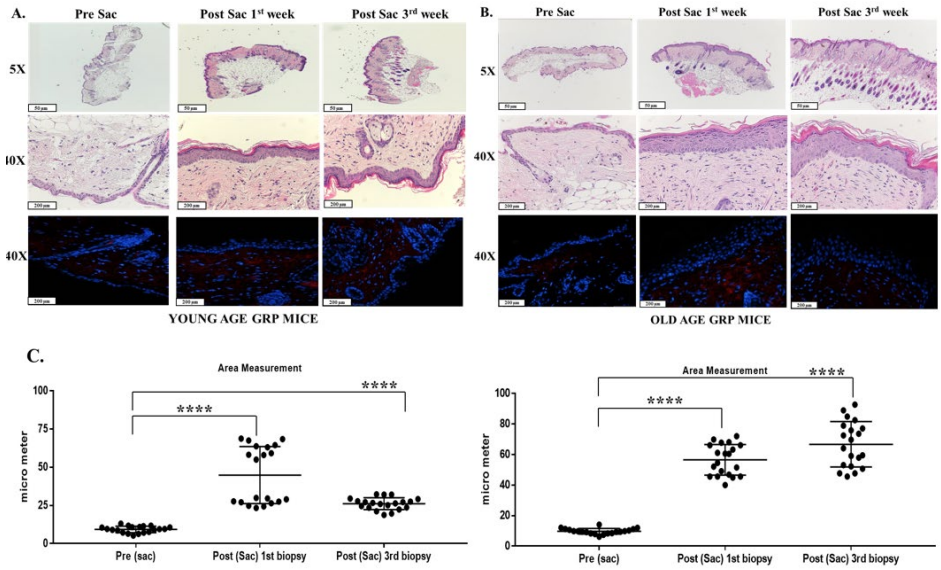




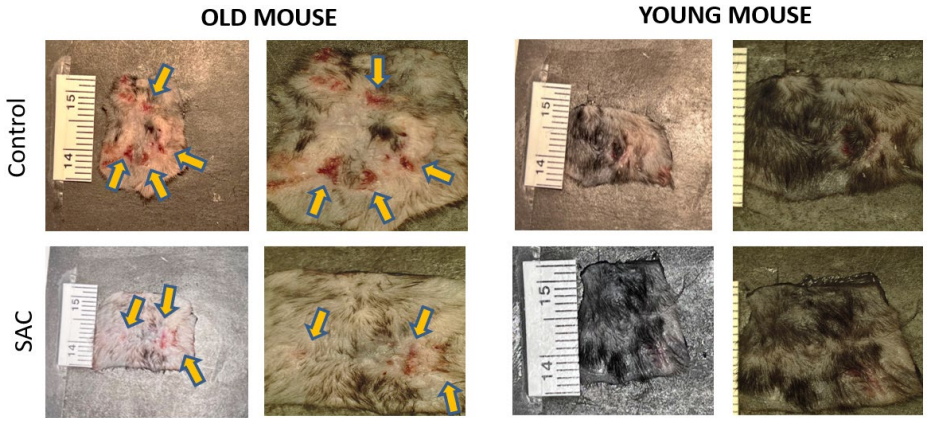
Measurement of Epidermis in Thick Areas

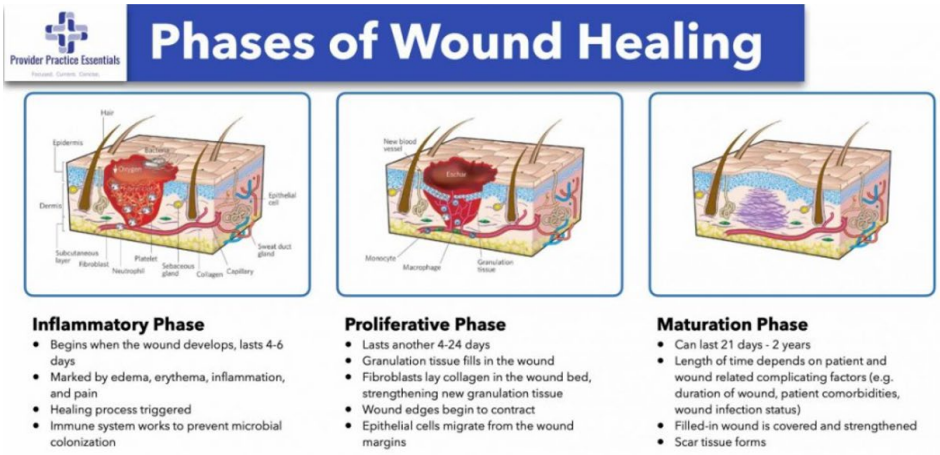


SAC topical cream application makes epidermal layer thick



SAC topical cream heals a biopsy wound better in older mouse.



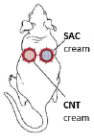


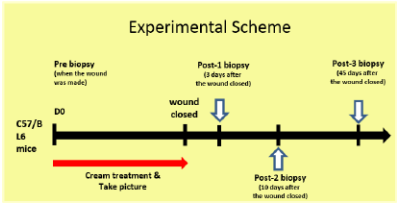
-Wound healing 3rd trial

-Previous Experimental Results

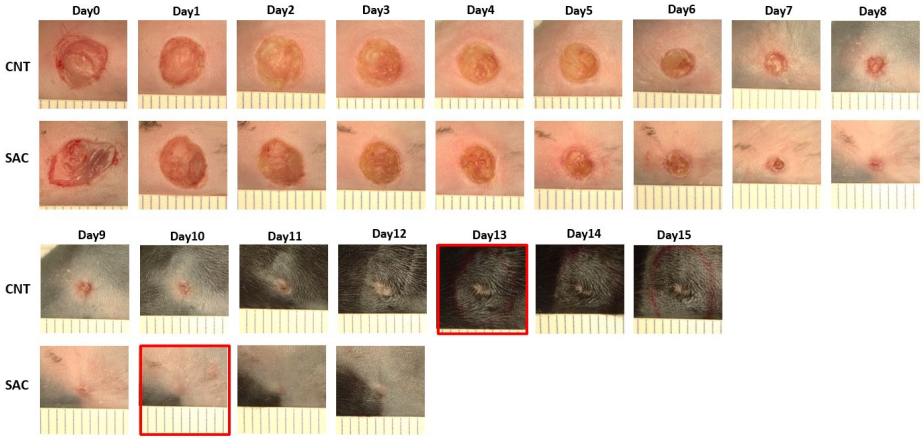
Our results demonstrated that the SAC cream treated group rapidly reduced the wound size in the early phase of healing and closed wounds earlier than the control cream treated group. Furthermore, the sizes of wound were consistently reduced in the SAC cream treated group while the ones in the control cream treated group varied.

1. Animal
   a. C57/BL6 mice (9months old, Female)
   b. Made a wound with 5mm punch biopsy (at 06/06/22)
   c. Pre biopsy(made wound) / Post-1 biopsy(after 3days closed) / Post-2 biopsy(after 10days closed) / Post-3 biopsy(after 45days closed, 08052022)
2. Group
   a. Mouse was single caged during the experiment.
3. Work
   a. Applied cream twice a day from the day made the wound
   b. Every day shaving, measurement and take a picture
   c. No cream was applied after the wound was closed.

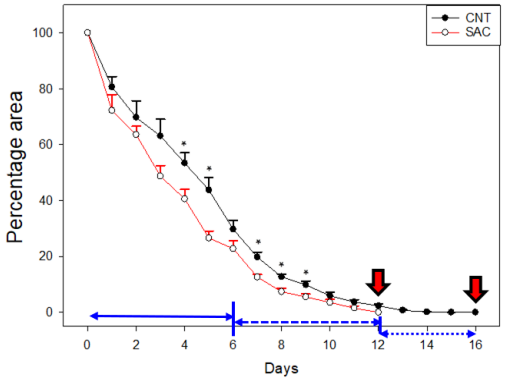




SAC treated wound closed faster than Control treated wound.

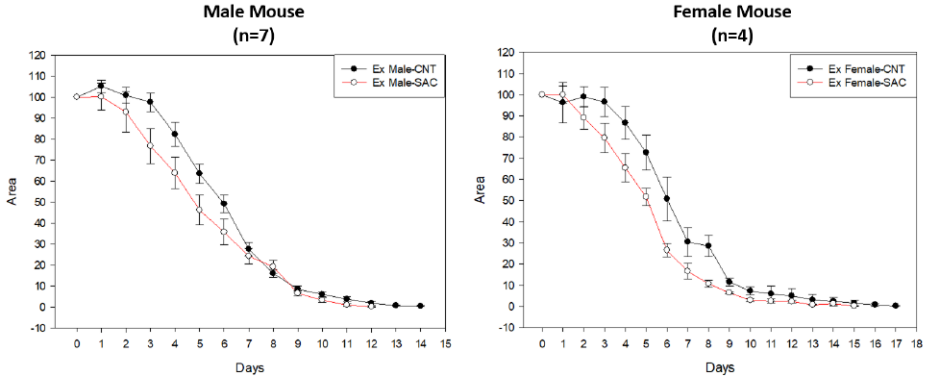


	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	100	85.8	96.3	81	62.9	58.9	36.7	21.2	14.4	8.9	4.5	4.8	1.2	0	0	0
SAC	100	88.9	79.3	62.5	48.8	27.3	25.2	10.2	4.4	3	0	0	0			



Difference	0.000	8.423	6.174	14.392	12.796	17.177	6.939	7.140	5.327	4.345	2.351	2.200	2.298	0.712	0.131	0.039	0.000

4th trial got significant variations due to method and scratches.



Sigma Anti-Bonding Calcium Carbonate (SAC) cream enhances the wound healing process.

JIHWAN SONG[1&2], Syed Hassan Mehdi[1], Jongsung Lee[3], Paul K Lee[4], Alex Lee[4], Yonggu Lee[2], and Donghoon Yoon[1]

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[2] Graduate School, Department of Pharmacy, Hanyang University, Ansan, Republic of Korea
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[4] CBHI (Calcium & Bone Health Institute, Canada), Coquitlam, British Columbia, Canada

I. INTRODUCTION

Sigma Anti-Bonding Molecule Calcium Carbonate (SAC) is a chemically modified calcium carbonate with a unique weak bonding structure that significantly increases the calcium absorbing rate in our body. It can influence environmental calcium levels and modulate calcium homeostasis *in vivo*. Wound healing is a calcium-mediated process. After cutaneous injury, hemostasis, inflammation, proliferation, and maturation occur during healing. Calcium regulates inflammatory cell infiltration, fibroblast proliferation, and keratinocyte migration. Collectively, we hypothesized that the topical treatment of SAC cream enhances wound healing.

II. MATERIALS AND METHODS

To study the effects of the SAC on wound healing, we took eight mid-aged (8~10-month-old) C57/BL6 female mice and singly housed them. The dorsum of each mouse was shaved using a clipper, then shaving cream. Two bilateral circular wounds were created on the dorsum using a 3- or 5-mm punch biopsy. The fur around the wound area was cut by scissors every morning. ~0.8mg of Control or SAC cream was applied to each circular wound twice a day for wound treatment. The SAC cream contains 1.6 mg/ml of SAC in a dermatology cream provided by the company. Cream application and wound measurement were performed for 14 days which is the time to close the wound by visual examination. The wound sizes (Area) were measured using CellSens software (Olympus Co.). In addition, skin biopsies were taken before the wound was created and after the wound closed. The skin biopsy was fixed in 4% paraformaldehyde and underwent hematoxylin and eosin (H&E) staining. The skin tissue was measured using a Zeiss AXIO Imager M2 microscope. In human dermal fibroblast cells, we also performed the cell proliferation assay using BrdU.

III. RESULT

As shown in Figure 1, our results demonstrated that the SAC cream treated group rapidly reduced the wound size in the early phase of healing and closed wounds early than the control cream treated group. Furthermore, the sizes of wound were consistently reduced in the SAC cream treated group while the ones in the control cream treated group varied.
Cell proliferation assay showed that SAC enhanced dermal fibroblast cell growth.

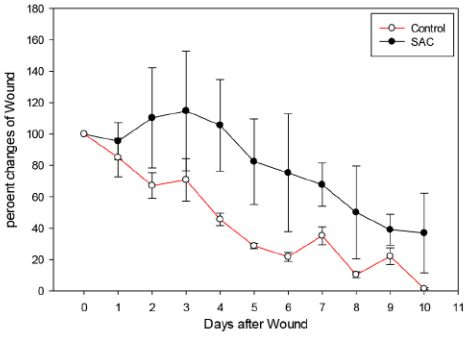


Figure 1: Wound Healing Rate

IV. CONCLUSION

We demonstrated that SAC cream improves wound healing by, at least in part, enhancing dermal fibroblast cell growth. The SAC cream can be used for cutaneous injury healing. The further study is necessary to identify the mechanism of action.

IV. ACKNOWLEDGEMENT

This research was supported by the Calcium and Bone Health Institute (CBHI; CBHI CRA C23992).

Increasing Ionic Calcium Inhibits Myeloma Cell Growth and its Progression in Mouse.

Syed Hassan Mehdi1, Alex Lee2, Paul K Lee2, Donghoon Yoon1

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Multiple myeloma (MM) is the second most common blood cancer, which forms in plasma cells in the bone marrow. MM-induced bone disease (MMBD) is one of the defining features of MM characterized by bone destruction and increased calcium level. We and others found that proteasome inhibitors (PIs) improve bone formation in MMBD, and a significant population of low-risk MM patients shows mineral deposition1-3. We developed a severe MMBD animal model4 and demonstrated that the bone anabolic agent promotes bone formation and delays MM progression5. Sigma Anti-Bonding Calcium Carbonate (SAC) is a physically modified calcium carbonate with a unique weak bonding. It acts as ionic calcium and significantly increases active ionized calcium in the body. We hypothesized that SAC inhibits the MMBD progression in the 5TGM1-induced MM mouse.

We divided the 8-12-week-old NOD SCID gamma mice into three groups: PBS, the SAC-Treated group before Transplant (TbT), and the SAC-Treated group after Transplant (TaT). was treated via intraperitoneal injection, BID, five days/week. Since myeloma cell engraftment/proliferation in mice occurs within two weeks from systemic injection, we gave two weeks before or after the transplant (TbT or TaT). The 1x106 luciferase-expressing 5TGM1 cells were injected into the mice via the tail vein. Mice were imaged weekly by IVIS imager to assess the myeloma progression and terminated when endpoint criteria were met. Survival curves were plotted, and the median survivals and statical difference between the two groups were tested by the Log-rank test using GraphPad Prism (San Diego, CA). At postmortem, the spine was removed, and ex vivo bone mineral content and bone mineral density were measured using a PIXI-mus bone densitometer with on-board PIXI-mus software (G.E.Lunar, Madison, WI, USA). For histomorphometric analyses, the

lumbar vertebrae (L1–L6) from the PBS and SAC-treated mice were scanned using a microCT40 (SCANCO Medical, Bassersdorf, Switzerland). The student's t-test was conducted using GraphPrism7.

We found that he median survival was 45 days in the PBS group, while 50 days in the TbT group and 49.5 days in the TaT group. The BL image analysis showed that myeloma slowly progressed in both treated group mice, and DEXA results demonstrated that bone mineral density and bone mineral content significantly increased in the spine of SAC-treated mice compared to the control mice. Trabecular thickness and bone volume density ($p < 0.05$) were significantly increased in the SAC-treated group at the lumbar spine compared to the PBS group.

Although our results showed that two weeks of SAC pretreatment in mice may not be sufficient to affect mouse survival, we demonstrated that the SAC treatment delays mouse myeloma progression in 5TGM1-induced NSG mice by promoting bone formation.

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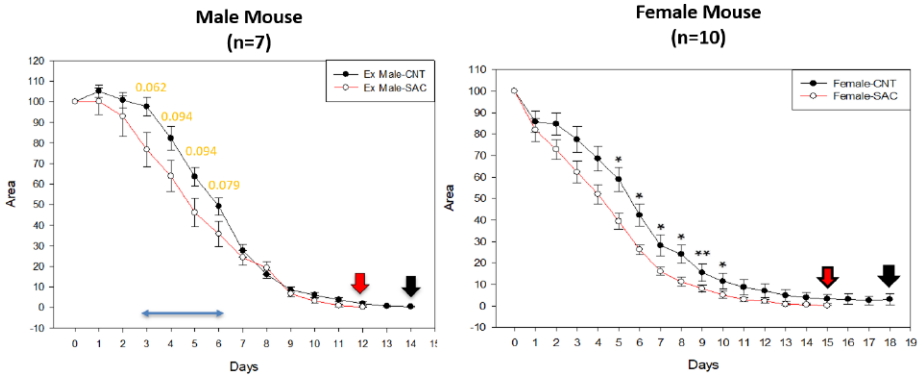
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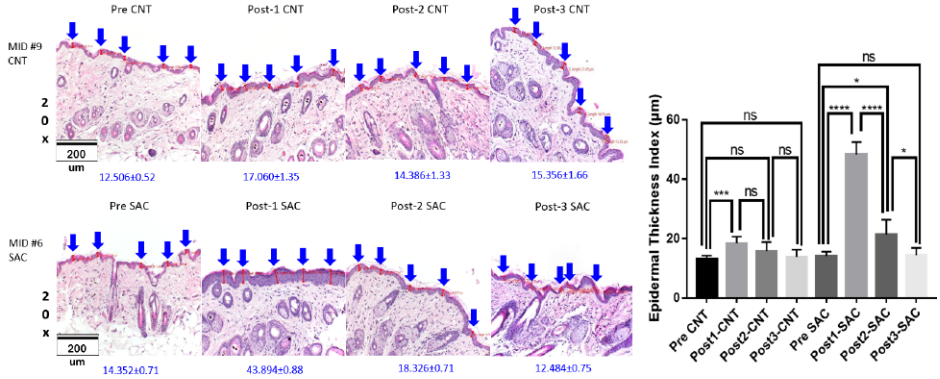
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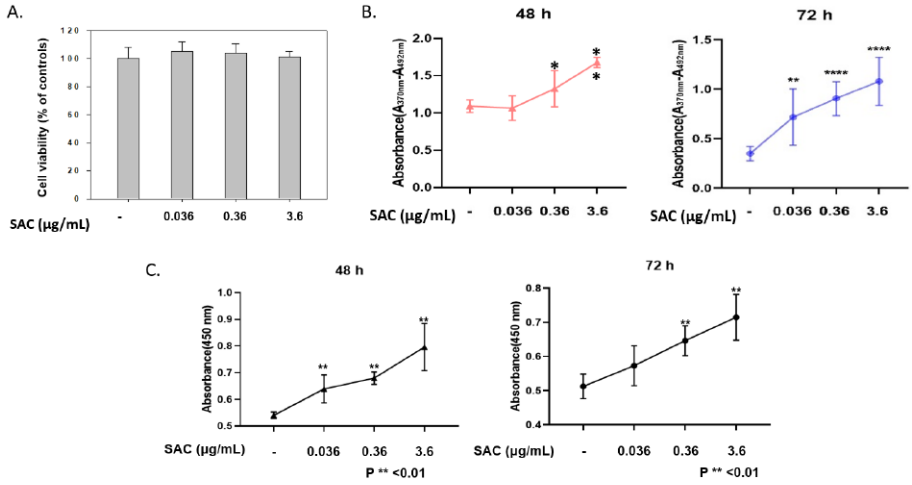
Pooled data show the same wound closure pattern between genders.

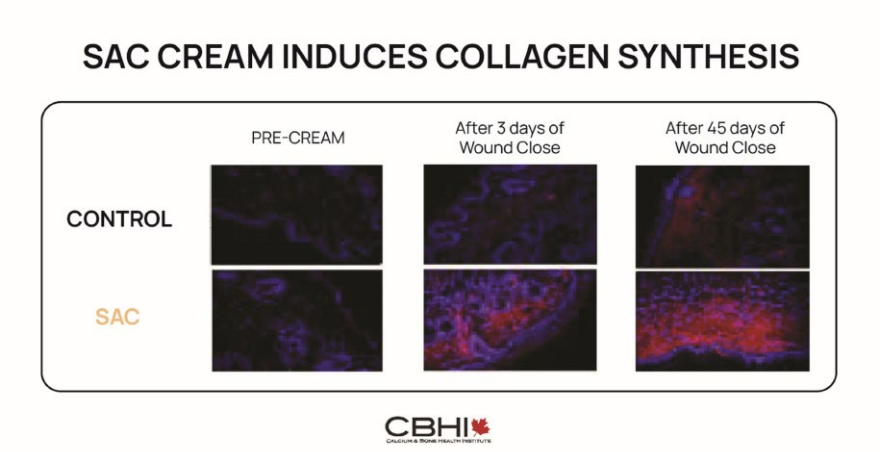


Epithermal thickness increases during SAC treatment, but it gets aging process again once sac treatment was stopped.



SAC induces no toxicity and induces human fibroblast and keratinocyte cell proliferation in a dose-dependent manner.



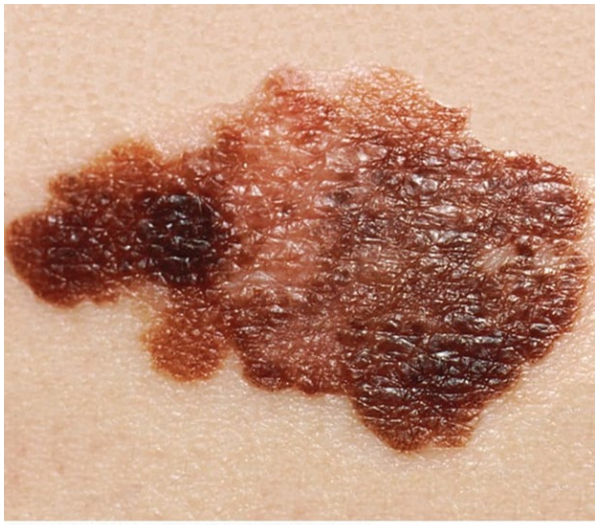


Melanoma, the most serious type of skin cancer, develops in the cells (melanocytes) that produce melanin the pigment that gives your skin its color. Melanoma can also form in your eyes and, rarely, inside your body, such as in your nose or throat.

The exact cause of all melanomas isn't clear, but exposure to ultraviolet (UV) radiation from sunlight or tanning lamps and beds increases your risk of developing melanoma. Limiting your exposure to UV radiation can help reduce your risk of melanoma.

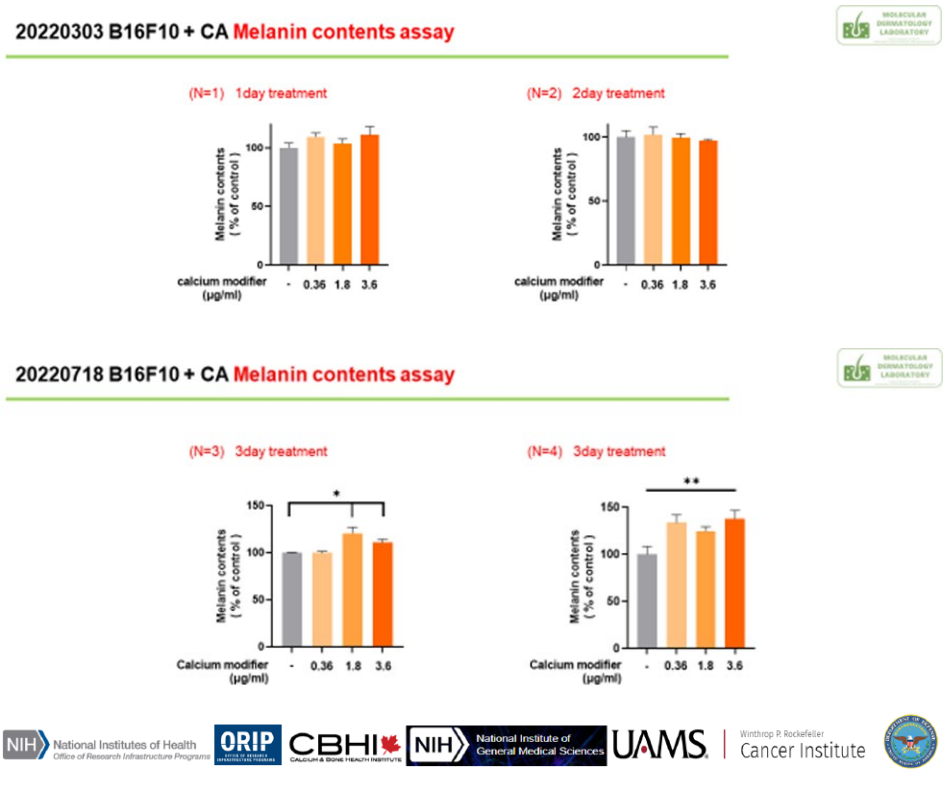
The risk of melanoma seems to be increasing in people under 40, es-

pecially women. Knowing the warning signs of skin cancer can help ensure that cancerous changes are detected and treated before the cancer has spread. Black people are far less likely to develop melanoma than non-Hispanic White people (at a rate of 1 per 100,000 compared to 30 per 100,000) due to the protection that melanin, the body's natural skin pigment, provides from damaging ultraviolet rays and if we can increase melanin content, we can protect skin cancers. We found that SAC calcium can increased melanin content in the skin cells.



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Melanoma



Funding Source

- NIH/CMIP, R21 OD026618, Principal Investigator, "In Situ Imaging of Collagen Degradation Activity in Multiple Myeloma and Lung Fibrosis Mouse Model"
- Calcium & Bone Health Institute (CBHI), Principal Investigator, "Study of Sigma Anti-bonding Calcium Role in Multiple Myeloma and Erythropoietin-Induced Osteoporosis."
- NIH/NIGMS, P20 GM125503, Project Leader (PI: Charles O'Brian), "Novel xenograft models to study racial differences in myeloma progression."
- UAMS VCRI Pioneer Award, Principal Investigator, "Bioactive Compounds from Herbal Medicine Prevent Multiple Myeloma via Gut Microbiota Changes"

- Department of Defense Idea Award, Co-Investigator (PI: Samantha Kendrick), "Targeting Unique DNA Structure to Repress Oncogenic Signaling in Lymphoma"

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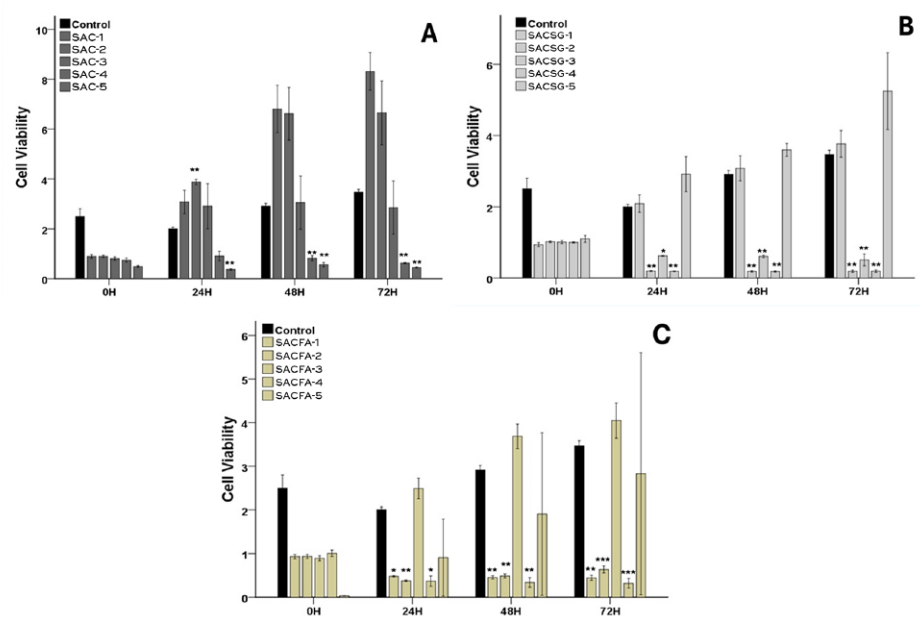
Hanyang Univ., Ansan, Korea
- Yong Gu Lee, PhD

SAC, SAC-SG and SAC-FA Invitro study report

Results effect of SAC-SG, SACFA, and SAC on the proliferation of breast cancer cells

By Dr.Server. Mersin University, Turkey.

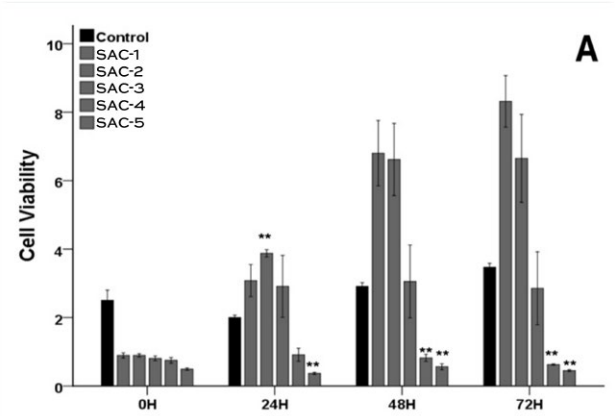
To investigate the effect of SAC-SG, SACFA, SAC at different dilutions on cell growth, MCF-7 cells were incubated for 72 hours at various dilutions (1/2, 1/4, 1/16, 1/32) and cell viability was measured using the MTT method.



Cell proliferation was measured both at pre-application (0H) and 24H, 48H and 72H after placing the SAC, SAC-SG, and SACFA of each compound in wells. Statistical significance was determined as follows: *P≤0.05, **P≤0.01, ***P≤0.001 when compared to the control.

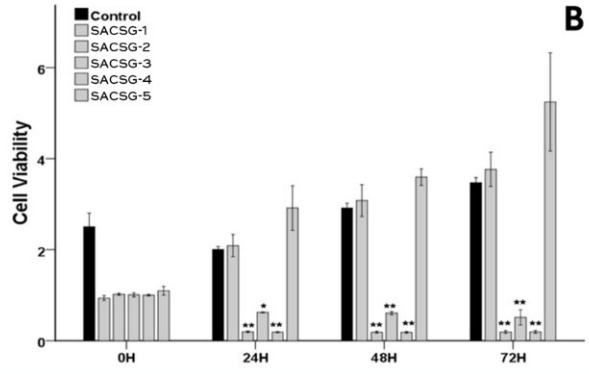
1: 1/32, 2: 1/16, 3: 1/8, 4: 1/4 5: 1/2 dilution

SAC Application



- Twenty-four hours after SAC application, statistically significant differences in cell proliferation were noted at the 1/16 and 1/2 dilutions compared to the control.
- Statistically significant decreases in cell viability were observed at the 1/2 and 1/4 dilutions after 48 and 72 hours.
- These results demonstrate that lower dilutions of SAC more pronouncedly affect cell viability over time.

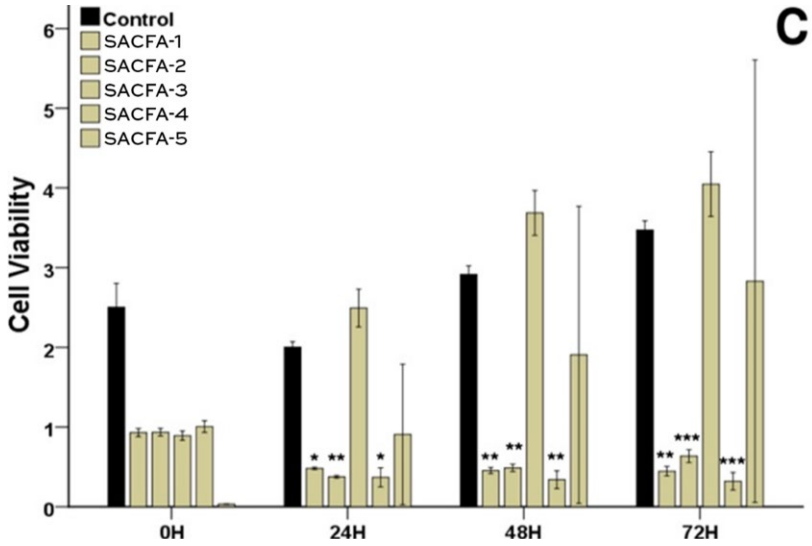
SAC-SG Application



Statistically significant reductions in cell proliferation were observed at the 1/16, 1/8, and 1/4 dilutions of SAC-SG across all measured time points (24, 48, and 72 hours).

This indicates a consistent and persistent decrease in cell viability at these concentrations over time.

SAC-SG Application



Statistically significant reductions in cell proliferation were observed at the 1/16, 1/8, and 1/4 dilutions of SAC-SG across all measured time points (24, 48, and 72 hours).

This indicates a consistent and persistent decrease in cell viability at these concentrations over time.

References:

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Research Article

A Potent Calcium Dietary Consumption Improves Bone Mineral Density in Asian Older Adults

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Abstract

Osteoporosis is a long-term bone disease that causes a gradual loss of bone density and increases the risk of bone fractures. The Asia Pacific region shows a higher prevalence of osteoporosis and associated fractures. Increasing calcium uptake is a well-known method to improve bone health. We took a potent calcium carbonate (Sigma Anti-bonding Molecule Calcium Carbonate, SAC). This clinical investigation evaluates the impact of a potent calcium carbonate supplement on Bone Mineral Density (BMD) in an Asian cohort. A total of 82 participants were enrolled/stratified into three groups based on T- score: normal (n=18), osteopenia (n=31), and osteoporosis (n=33). T-scores, BMD, and speed of sound (SOS) were measured at baseline and following ~eight months of SAC intervention. All measurements were conducted at the distal radius, a clinically relevant site for assessing peripheral bone density.

After intervention, T-scores improved significantly (from -2.0 to -0.85, $p<0.0001$) along with increased BMD (from 0.95 to 1.10 g/cm^2, $p<0.0001$) across all ages and genders. SAC treatment also significantly increased SOS values from 3,946 to 4,079 ($p < 0.0001$). 65% of osteopenia participants (20/31) improved to normal after treatment, while 64% of osteoporosis participants (21/33) moved to osteopenia. Remarkably, 12% of baseline osteoporosis participants (4/33) transitioned into the normal bone density range. We found that a dietary supplement of potent calcium, SAC, leads to significant improvements in bone health in Asian individuals, a population with higher susceptibility to osteoporosis. These results suggest that SAC may be a useful option to attenuate osteoporosis.

Keywords: Osteoporosis; Osteopenia; Bone mineral density; Sigma Anti-bonding Molecule Calcium Carbonate (SAC)

Introduction

Osteoporosis is a chronic skeletal disorder defined by reduced bone mass and microarchitectural deterioration, which increases bone fragility and fracture risk [1]. Fractures due to osteoporosis are a major source of morbidity and mortality in older adults, imposing a substantial socioeconomic burden worldwide [2]. As the most common metabolic bone disease, osteoporosis is progressive and associated with pain, disability, and loss of independence [3,4]. Large population-based studies have shown excess mortality in individuals with osteoporosis compared with the general population, even when treated, partly due to fracture-related complications [5].

The prevalence of osteoporosis is rising with global population aging. It affects ~10% of the general population and up to 30% of post-menopausal women [6]. In Asia and other high-income regions, including Japan, South Korea, Taiwan, Singapore, and Australia, the burden of osteoporotic fractures is particularly significant [7]. Racial and ethnic differences are evident; for example, Asian adults often have lower bone mineral density (BMD) than White counterparts, largely due to differences in skeletal size and body composition [8,9].

Pharmacological therapies, particularly bisphosphonates, are widely prescribed for patients at high fracture risk. Meta-analyses confirm that bisphosphonates reduce overall fracture incidence (odds ratio 0.62, $p<0.001$). Among several medication options, bisphosphonates are frequently prescribed as first-line therapy for patients with high fracture risk [10,11]. However, these agents do not fully halt disease progression, and residual fracture risk remains high. Adjunctive calcium and vitamin D supplementation are often recommended, although the optimal regimen remains uncertain [12]. Consequently, more effective and targeted treatment options for osteoporosis are urgently needed. Additionally, research results showed that even if osteoporosis is treated, the mortality rate and fractures still increase [5,13].

Calcium carbonate is a common supplement derived from natural sources such as oyster shell. Sigma Anti-Bonding Molecule Calcium Carbonate (SAC) is a modified form with weakened sigma anti-bonding, yielding higher bioavailability than conventional calcium carbonate [14]. In animal studies, SAC improved bone density by modulating osteocalcin, collagen, and mineral deposition without adverse effects [15]. Despite promising preclinical evidence, its efficacy in humans has not been evaluated. This study aimed to assess the effect of SAC supplementation on bone mineral density in an Asian population.

Materials and Methods

Study Design and Participants

This retrospective study was conducted at a medical clinic in Vancouver, Canada. Participants were recruited through newspapers, social media, and word-of-mouth advertisements. Participants received 7.6 mg SAC daily, administered as 5 ml of solution diluted in 500 ml of water per 70 kg body weight, taken twice daily on an empty stomach or between meals. Individuals were excluded if they had: 1) received osteoporosis treatment within the past year; 2) significant hepatic impairment (cirrhosis, active hepatitis); 3) impaired renal function or history of kidney/urinary stones; 4) inability to perform light exercise or maintain regular meals; 5) secondary osteoporosis requiring other treatments. After providing written informed consent, all participants underwent baseline bone density assessment using ultrasound and were prescribed SAC. The protocol was approved by the Institutional

Review Board (Protocol no. MCC23681).

Bone Density Measurement

Bone density was assessed with a portable ultrasound device (BeamMed™ MiniOmni™). Daily calibration was performed using a standard block. Measurements were taken from the distal radius with the participant seated and forearm resting on the device [16].

Data Processing and Transformation

Ultrasound-derived speed of sound (SOS) values were converted to BMD and T-scores using Asian population reference standards from the World Health Organization (WHO) and regional epidemiological studies. Data conversion was validated to ensure consistency and minimize errors. The standard deviation (SD) of BMD in individuals compared to young adults is referred to as the T score. Based on this score, individuals were classified as normal (T-score > −1.0), osteopenia (−2.5 < T-score < −1.0), or osteoporotic (T-score ≤ −2.5) [4]. A T-score of 0 was defined as a BMD value of 1.2 g/cm^2.

Sample Size Calculation

The sample size was calculated using G*Power 3.1 (Heinrich-Heine-Universität Düsseldorf, Germany) to ensure adequate statistical power (0.80) at an alpha level of 0.05. For the chi-square test, an effect size of 0.6 (medium to large, based on prior literature) and 2 degrees of freedom were assumed, totalling 82 participants.

Statistical analysis

The efficacy of SAC was evaluated based on changes in bone mineral density (BMD), T-score, and speed of sound (SOS) of the distal radius after approximately 8 months of treatment.

Baseline Characteristics

Baseline characteristics of the study participants were compared across three clinical groups (normal, osteopenia, and osteoporosis). Numerical variables were analyzed using one-way analysis of variance (ANOVA) for comparisons across the three groups. Categorical variables were analyzed using the chi-square test or Fisher's exact test when expected cell counts were less than 5.

Within-Group Changes: Changes in BMD, T-score, and SOS from baseline to post-treatment within the same group were analyzed using a paired t-test.

Between-Group Comparisons: Between-group differences in BMD changes after treatment were analyzed using analysis of covariance (ANCOVA), with baseline BMD as a covariate. For T-score and SOS, similar ANCOVA models were applied, with baseline T-score and SOS as respective covariates. To address multiple comparisons across BMD, T-score, and SOS, a Bonferroni correction was applied, adjusting the significance level to $p < 0.0167$ (0.05/3).

All statistical analyses were performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA).

Results

Baseline characteristics

A total of 82 participants participated in this study. Table 1 presents the baseline characteristics of the participants, which showed no significant differences in the measured parameters except average age. The average age was approximately 54 for normal, 62 for osteopenia, and 64 for the osteoporosis group ($p<0.0001$).

Variables		Normal (n=18)	Osteopenia (n=31)	Osteoporosis (n=33)	p value
Age ± SD		54.0 ±9.7	62.3 ± 9.7	63.6 ± 7.1	<0.0001
Gender	Male	8	8	5	0.07
	Female	10	23	28	
Weight ± SD (kg)		60.5 ± 11.8	58.5 ± 8.4	58.2 ± 5.6	0.65
Height ± SD (cm)		163.0 ± 6.0	161.3 ±6.8	157.9 ± 4.9	0.10
BMI ± SD		22.6 ± 3.2	22.4 ± 2.3	23.0 ± 1.9	0.86
Intervention ± SD (days)		279 ± 223	215 ± 249	273 ± 272	0.59

Table 1: Characteristics of participants.

Changes of T-score, SOS, and BMD by clinical group, gender, and age group

T-scores, SOS, and BMDs were measured at baseline and ~8 months of SAC. As expected, the mean differences from baseline were statistically significant among clinical groups ($p<0.0001$, Figure 1). After treatment, SAC treatment significantly increased T score (-2.01 vs. -0.85, $p<0.0001$, Table 2), SOS (3,946 vs. 4,079, $p<0.0001$, Table 2), and BMD (0.95 vs. 1.10 g/cm2, $p<0.0001$, Table 3) regardless of gender and age groups. These improvements were more pronounced among participants with osteoporosis and in male subjects ($p<0.0001$) (Figure 1D). The efficacy of SAC was more evident in the men's group overall (Figure 1D, Table 2).

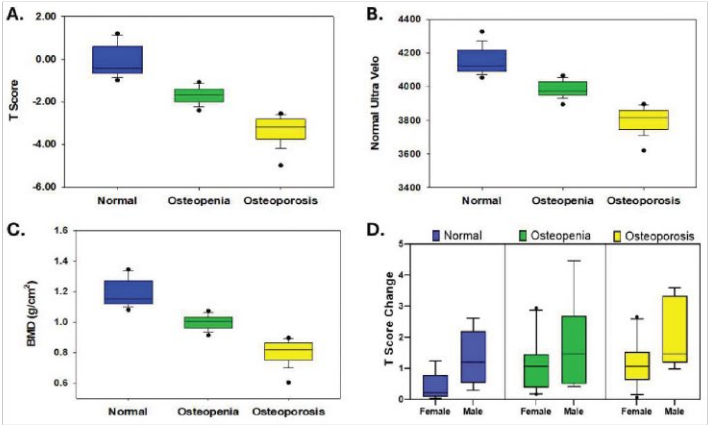


Figure 1: Baselines of T-Score, SOS, BMD, T Score Changes with 10th, 25th, median, 75th, 90th values in clinical groups. A: Median of T-scores are -0.11 (normal) vs -1.68 (osteopenia) vs. -3.34 (osteoporosis, $p<0.0001$). B: Median of SOS Scores are 4,153 vs 3,983 vs 3,798 ($p\leq0.0001$), and C: Median of BMD are 1.19 vs 1.00 vs 0.80 ($p<0.0001$). D. T score change after SAC treatment. Male and osteoporosis groups showed higher efficacy than female and other clinical groups ($p<0.0001$).

	Gender	values	number	p value
Age ± SD	Female	58.0 ± 10.2	61	0.858
	Male	58.5 ± 11.0	21	
SOS ± SD (%)	Female	2.91 ± 0.02	61	0.004
	Male	4.75 ± 0.03	21	
T-Score ± SD (%)	Female	51.8 ±45.3	61	0.001
	Male	262.0 ± 494.2	21	
BMD ± SD (%)	Female	13.6±10.2	61	0.035
	Male	20.0±15.9	21	
Menopause				
Age ± SD	Yes	61.3 ± 6.23	48	<0.0001
	No	43.5 ± 8.89	13	

SOS ± SD (%)	Yes	3.16 ± 2.04	48	0.075
	No	2.00 ± 2.04	13	
T-Score ± SD (%)	Yes	48.6 ±40.7	48	0.288
	No	63.8 ± 59.7	13	
BMD ± SD (%)	Yes	15.1±10.2	48	0.028
	No	8.11±8.34	13	

Table 2: Changes of T-score, SOS, and BMD by gender or menopausal status.

	Gender	T-score			
		Baseline ± SD	**After ± SD**	**N**	**p value**
All		-2.0±1.38	-0.85±1.51	82	<0.0001
Normal	All	-0.11±0.69	0.70±1.09	18	<0.0001
Normal	Female	-0.22±0.76	0.21±0.63	10	0.01
Normal	Male	0.03±0.62	1.33±1.26	8	0.004
Osteopenia	All	-1.68±0.37	-0.46±1.11	31	<0.0001
Osteopenia	Female	-1.69±0.40	-0.63±0.89	23	<0.0001
Osteopenia	Male	-1.64±0.37	0.04 ±1.56	8	0.013
Osteoporosis	All	-3.34±0.66	-2.06±0.98	33	<0.0001
Osteoporosis	Female	-3.37±0.69	-2.23±0.91	28	<0.0001
Osteoporosis	Male	-3.20±0.48	-1.10±0.78	5	0.015
SOS					
All		3946±153	4079±164	82	<0.0001
Normal	All	4153±79	4246±113	18	<0.0001
Normal	Female	4163±87	4212±71	10	0.012
Normal	Male	4140±71	4289±144	8	0.004
Osteopenia	All	3983±48	4123±122	31	<0.0001
Osteopenia	Female	3995±44	4116±100	23	<0.0001
Osteopenia	Male	3949±42	4141±180	8	0.13
Osteoporosis	All	3798±76	3946±105	33	<0.0001
Osteoporosis	Female	3804±79	3934±104	28	<0.0001
Osteoporosis	Male	3769±56	4011±89	5	0.015
BMD					
All		0.95 ±1.65	1.10 ± 0.18	82	<0.0001
Normal	All	1.19±0.08	1.28±0.13	18	<0.0001
Normal	Female	1.17±0.09	1.22±0.08	10	0.01
Normal	Male	1.20±0.07	1.36±0.15	8	0.004
Osteopenia	All	1.00±0.05	1.14±0.13	31	<0.0001
Osteopenia	Female	1.00±0.05	1.12±0.11	23	<0.0001
Osteopenia	Male	1.00±0.04	1.20±0.19	8	0.013

Osteoporosis	All	0.80±0.08	0.95±0.12	33	<0.0001
Osteoporosis	Female	0.80±0.08	0.93±0.11	28	<0.0001
Osteoporosis	Male	0.82±0.06	1.07±0.09	5	0.015

Table 3: T-score, SOS, BMD changes by gender and clinical groups.

The status of menopause is critical to bone health. As expected, participants with menopause showed significantly lower values of SOS (3,888 vs. 4,110, p<0.0001), T-score (-2.64 vs. -0.69, p<0,0001), and BMD (0.88 vs. 1.12, p<0.0001). The percentage increase in BMD after treatment was significantly higher in the menopausal group compared to the non-menopausal female group (15.1% vs. 8.1%, p=0.028). This association was not observed in changes of SOS (p=0.075) and T-score (p=0.288, Table 2).

Aging is a well-established risk factor for osteoporosis and plays a critical role in bone health deterioration. Significant inverse relations between age and bone health were observed on T-score (Pearson correlation with age, -0.41, p<0.0001), SOS (Pearson correlation with age, -0.43, p<0.0001), and BMD (Pearson correlation with age, -0.41, p<0.0001) (Figure 2). Efficacy of SAC was observed in all age groups. Therefore, there is no significant difference in efficacy among age groups (SOS change p=0.114, T-Score change p=0.324, BMD change p=0.124) (Figure 3).

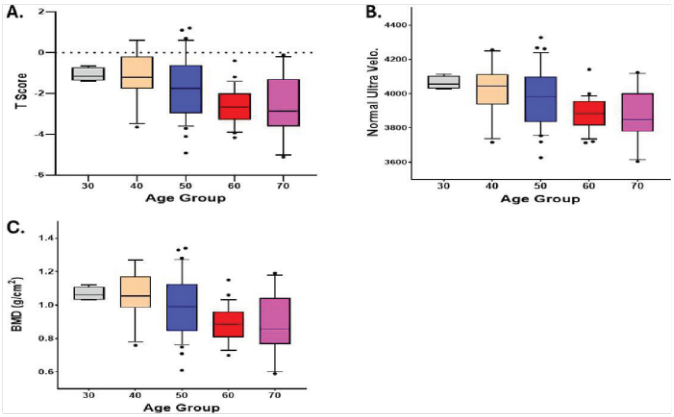


Figure 2: Inverse relationship between age and bone health. A: T-score (p<0.0001), B: SOS (p<0.0001), C: BMD (p<0.0001)

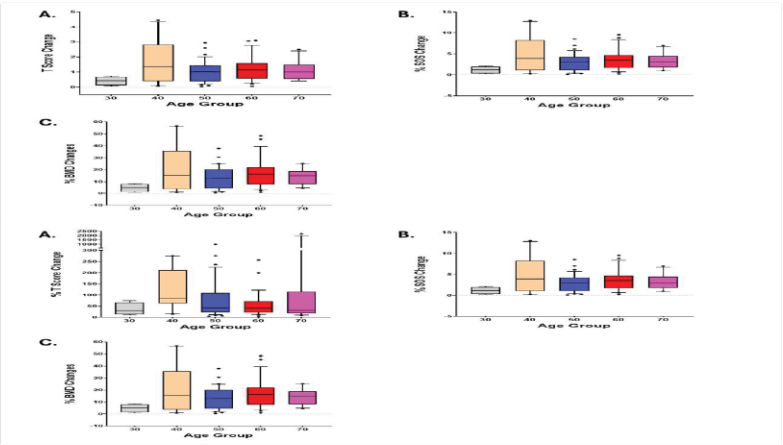


Figure 3: Efficacy of SAC by age groups. Efficacy of SAC was observed in all age groups. There is no significant difference in efficacy among age groups. A: T-score change p=0.324, B: SOS change p=0.114, and C: BMD change p=0.124.

Among 82 participants, 65% of the osteopenia participants (20/31) improved to the normal range after 8 months of treatment, while 64% of participants with osteoporosis (21/33) were reclassified into the osteopenia group. Moreover, 12% of osteoporosis participants (4/33) improved to the normal range (Figure 4).

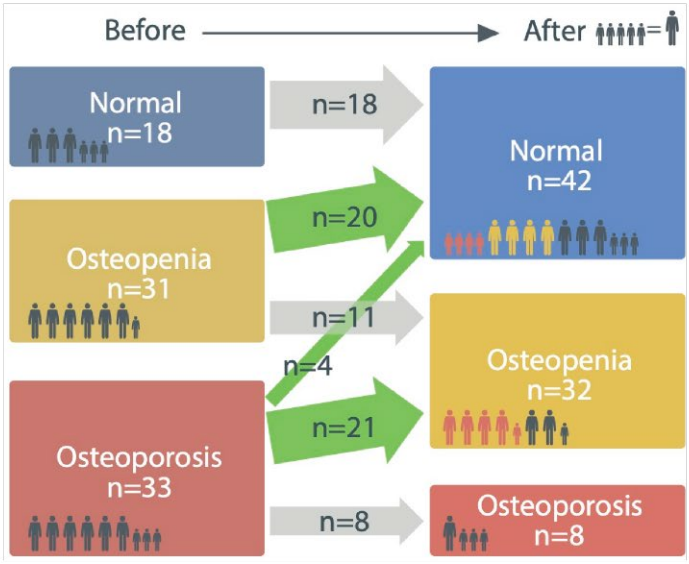


Figure 4: Distribution of clinical group before and after treatment based on T-score. 65% of participants with osteopenia (20/31) were improved to the normal group after treatment, while 64% of participants with osteoporosis (21/33) became the osteopenia group. Notably, 12% of osteoporosis participants (4/33) improved to the normal range.

Discussion

In this study, we observed that 8 months of daily SAC supplementation significantly improved BMD, T-score, and SOS in both men and women across all age groups and all clinical groups. To the best of our knowledge, this is the first study to investigate the effects of SAC in humans.

Calcium plays a crucial role in many physiological processes. In mammals, serum calcium is tightly maintained in a narrow range (1-1.3 mM) [17]. Normally, dietary calcium is absorbed via the lumen with the aid of Vitamin D, but it is immediately bound to albumin, becoming physiologically inactive.

The SAC is a calcium carbonate formulation characterized by weak sigma anti-bonding interaction with water molecules, forming $Ca(H_2O)_6$. Due to its loosely bound molecules, it enables absorption into the bloodstream without vitamin D, staying bioavailable ionized calcium. Such elevation of bioavailable calcium in the body resulted in an increase in extracellular calcium. In the bone environment, these extracellular Ca2+ signals interact with BMP and promote osteoblast differentiation, resulting in bone formation from the bone marrow-mesenchymal stem cells and increasing BMD, depicted in Figure 5 [18]. Furthermore, it is likely to reduce parathyroid hormone secretion, mitigating calcium loss and suppressing osteoporosis progression. As shown in the animal model [15]. We hypothesized that oral consumption of SAC increases serum bioactive calcium without or with minimal interruption of total calcium homeostasis and improves patients' osteoporosis status.

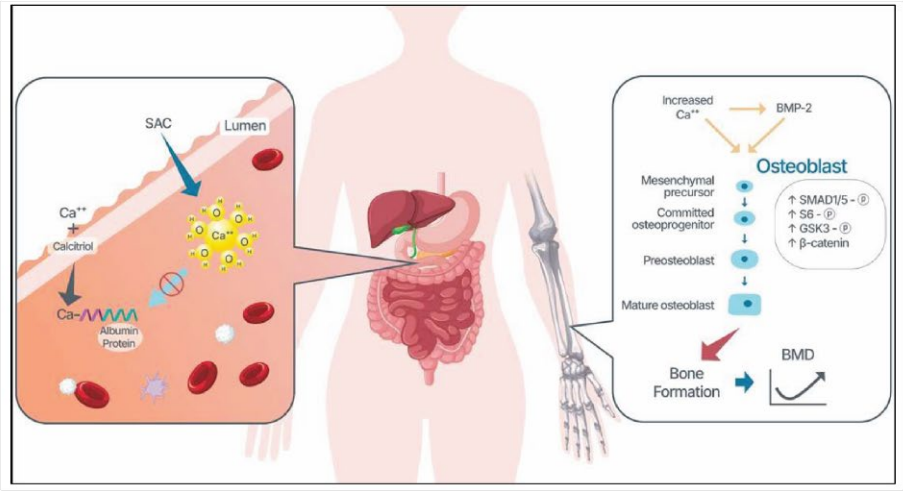


Figure 5: Proposed mechanism of how SAC improves BMD. Due to a weak intermolecular bonding of SAC with water molecules, forming $Ca(H_2O)_6$, the SAC enables absorption into the bloodstream without vitamin D, staying bioavailable as ionized calcium. It elevates extracellular calcium in the bone microenvironment. This extracellular calcium and BMP-2 cooperatively promote the osteoblast differentiation/activity, increasing bone formation.

SAC differs from conventional calcium carbonate in its weak sigma anti-bonding, which enhances solubility and absorption, allowing bioactive calcium to remain in circulation without reliance on vitamin D [14]. Increased extracellular calcium may stimulate osteoblast differentiation via BMP signalling and suppress parathyroid hormone, thereby reducing bone resorption [15, 18].

Based on a 16% increase in BMD by SAC treatment, a significantly reduced fracture risk was anticipated in participants (Table 3) as previous studies demonstrated an inverse relationship between BMD change and fracture risk [19]. A meta-analysis study reported that a calcium supplement is effective in increasing BMD and reducing fractures of the bone [19, 20].

Menopause is one of the established risk factors for osteoporosis. It leads to low bone density and progresses into a decrease in BMD [21]. In fact, osteoporosis affects 30% of post-menopausal women [6]. In our study, the percentage of BMD after treatment of the menopausal group showed higher improvement compared to the non-menopausal female group (15.15% vs. 8.11%, p=0.028). Estrogen deficiency due to menopause can, in part, explain osteoporosis. Low estrogen levels lead to the outflow of calcium from the bones. This process lowers parathyroid hormone level and calcium absorption, contributing further to calcium loss from the bone [22, 23].

Our findings should be interpreted cautiously. First, the small sample size and retrospective design limit generalizability. Second, ultrasound, though cost-effective and widely available, is less established than DXA for guiding osteoporosis management. Third, most participants were East Asian, so findings may not extrapolate to other populations.

Despite these limitations, SAC demonstrated consistent efficacy and safety, making it a promising candidate for osteoporosis prevention and treatment. Larger, prospective randomized controlled trials with DXA-based endpoints and fracture outcomes are warranted.

Asian populations are more susceptible to osteoporosis than individuals of other racial backgrounds. Based on BMD, 32 % of 65-79 years old, and 18% of 50-64 years old Asian females have osteoporosis [24]. In addition, low calcium intake, a small body frame, and low physical activity contribute to a higher risk.

A larger, well-designed study with higher statistical power and a longer follow-up period is needed to further validate our findings. We are aware of the limitations of this study. Although ultrasound measurement costs less and has been proposed as a potential alternative to DXA measurements of bone mineral density (BMD), there is still uncertainty in the incorporation of ultrasound in the clinical management of osteoporosis. However, ultrasound is more widely used in clinics. Significant progress has been made, and new studies are promising. The second limitation is a small sample size, which limits generalizability. Finally, most participants are of East Asian descent. Therefore, these findings may not be extrapolated to other populations. Despite these limitations, SAC demonstrated consistent efficacy and safety, making it a promising candidate for osteoporosis prevention and treatment. Larger, prospective randomized controlled trials with DXA-based endpoints and fracture outcomes are warranted.

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All authors are to declare non-financial interests in relation to the submitted work.

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Q. What is the fundamental solution for age-related chronic degenerative diseases?

Calcium homeostasis plays a critical role in maintaining our health, particularly in relation to bone health and DNA mutation as a cause of cancer. Adequate calcium levels in serum and cells are essential for the formation and strength of bones, as well as for functions such as muscle contraction, blood vessel regulation, nerve signaling, hormone functions and DNA replication.

Q. What happens if calcium homeostasis breaks down?

When calcium homeostasis breaks down, it can have significant implications for various physiological processes. Disruption of calcium balance may lead to dysfunctions in p53, a tumor suppressor protein, potentially increasing the risk of cancer. Furthermore, imbalances in calcium ions can affect mitochondrial function, which plays a critical role in cellular energy production. These disturbances in calcium homeostasis and mitochondrial function have been associated with chronic degenerative and neurodegenerative diseases, including astrocyte dysfunction, synaptic dysfunction, Alzheimer's disease, Parkinson's disease, ALS, Huntington's disease, diabetes, arrhythmia, epilepsy, ADHD, autism, DNA mutation and more.

Q. What is the SAC®?

In the human body, there are over 35 trillion cells that undergo continuous replication throughout our lifespan. However, as we age, the rate and function of cell replication gradually decline. This decline is often accompanied by disruptions in calcium homeostasis, leading to mitochondrial dysfunction and DNA mutations. When calcium levels become imbalanced inside and outside the cells, it impairs proper cellular function, potentially resulting in chronic neurodegenerative diseases such as cancer and osteoporosis.

Maintaining a balance in calcium levels is crucial to preserving cellular health. In this regard, I am confident that SAC® (Sigma Anti-bonding Calcium) can serve as a fundamental key in the treatment of chronic degenerative diseases, including cancer.



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