The Effect of Trace Elements in Experimental Colon Cancer

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PREFACE

The book "**Effect of trace elements in experimental colorectal cancer**" is the result of the work of three outstanding professionals: Dr. Rosa María Bergoc, Dr. Juan Carlos Perazzo, both basic researchers dedicated to university teaching, and Dr. Ernesto J.V. Crescenti, a medical doctor specialized in gynecology, surgery, and oncology. They present their results on the effects of the essential oligoelements zinc, selenium, and manganese (OLM) on an animal model of chemically induced colorectal cancer (CRC). CRC is the third most common cancer worldwide and the second leading cause of cancer death, resulting in more than 900,000 deaths per year according to the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO). It is caused by the progressive accumulation of genetic and epigenetic alterations that lead to the tumorigenic transformation of normal colonic epithelium. The number of cases is increasing annually, and there are no effective therapies, highlighting the need for new therapeutic approaches. Solid results described herein show the mechanisms of action of OLM trace elements in the CRC model, demonstrating that OLM delay the appearance of tumors and reduce their incidence.

Trace elements are essential for biological, chemical, and molecular cellular functions as they mediate vital biochemical reactions by acting as cofactors for many enzymes and regulating transcription factors. In addition, trace elements are determinants in the regulation of cellular pathways mediated by matricellular proteins, which are key molecules necessary for both carcinogenesis and its inhibition. Likewise, the authors discuss for the first time in this model the involvement of certain cell death processes, such as autophagy and the non-apoptotic cell engulfment process, entosis.

Based on the results presented in this book, and in accordance with previously published results, the authors emphasize the role of the extracellular matrix as the medium through which the processes of growth, invasion, differentiation, and cell death are regulated. Moreover, in the last chapter dedicated to the conclusions, the effect of OLMs on these interrelationships is also discussed.

This book offers the reader promising basic and preclinical findings that give hope for the potential therapeutic use of OLM in CRC patients. These findings can be extrapolated to other tumor types, considering the beneficial effects of trace elements previously reported by the authors and other researchers in several cancer models. Additionally, the book opens several other avenues of research. Given that OLMs positively regulate immunity and negatively regulate tumor development and growth, one potential research direction would be to determine whether the antitumor action of OLMs is mediated by an increase in antitumor immunity. On the other hand, in the tumor microenvironment (TME), the intricate interaction between the ECM and immune cells constitutes a complicated network that plays a critical role in the mechanisms underlying tumorigenesis, progression, and metastasis. Since OLMs significantly contribute to the functionality of the ECM and can modulate the immune landscape of the TME, another possible research route is to evaluate the action of OLMs in these interactions. Once we delve into the mechanisms and add to the strong evidence presented in this book, we will surely be closer to making the therapeutic use of OLM more than a simple promise.

Prof. Dra. Graciela A. Cremaschi, Ph.D.

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CHAPTER 1

TRACE ELEMENTS

R.M. BERGOC, J.C. PERAZZO
AND E.J.V. CRESCENTI

Introduction

Nutrient and Trace Elements

Food contains nutrients that play a direct or indirect role in the activities an organism needs to fulfill its vital functions. According to the importance of their participation in the body's metabolic reactions, nutrients can be non-essential or essential. The former are not vital for the body, while the latter are. For humans, the latter correspond to essential fatty acids, essential amino acids, some vitamins, and some minerals. Of course, water and oxygen are also essential for human survival.

On the other hand, from a nutritional point of view, nutrients can be classified as macronutrients and micronutrients according to the amount required. Macronutrients are carbohydrates, proteins, lipids, and fats in general; they are required in high quantities and provide energy. Micronutrients, vitamins, and minerals are required in smaller quantities.

In human nutrition, daily requirements for macronutrients are in the order of grams, while those for micronutrients are generally less than 100 milligrams. Micronutrient deficiencies affect several million people worldwide and are the cause of numerous diseases. The most important deficiencies are related to iodine, zinc, calcium, selenium, and vitamins such as A, C and the B complex (World Health Organization, WHO).

Minerals can be classified into macrominerals, such as calcium, sodium, chlorine, phosphorus, magnesium, potassium, and sulfur, and microminerals. Microminerals or trace elements, such as zinc, iodine, selenium, iron, and copper, are essential and specific components of the structure of various molecules and participate in a large number of biological processes, such as the functioning of the immune system.

Consequently, they are of fundamental importance in health (Morris and Mohiuddin 2024).

Nutrients

Nutrients play direct and indirect roles in the activities an organism needs to fulfill its vital functions. According to the importance of their participation in the body's metabolic reactions, nutrients can be non-essential or essential. The first are not vital for the body, while the latter are. For humans, the latter correspond to essential fatty acids, essential amino acids, some vitamins, and some minerals. Of course, water and oxygen are also essential for human survival.

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In human nutrition, daily requirements for macronutrients are in the order of grams while those for micronutrients are in order of milligrams or microgram. Micronutrient deficiencies affect several million people worldwide and are the cause of numerous diseases. The most important deficiencies are related to iodine, zinc, calcium, selenium and vitamins such as A, C and those of the B complex (WHO, World Health Organization).

Minerals can be classified into macrominerals as calcium, sodium, chlorine, phosphorus, magnesium, potassium, and sulfur, or microminerals.

Microminerals or trace elements, such as zinc, iodine, selenium, iron, copper, are essential and specific components of the structure of various molecules and participate in a large number of biological processes, such as the functioning of the immune system. Consequently, they are of fundamental importance in health (Erickson, Medina, and Hubbard 2020; Razzaque and Wimalawansa 2025). Adequate micronutrients are required, for example, to prevent damage of cells participating in innate immunity

Trace elements

Trace elements, also called oligoelements (oligo: little in Greek), are important chemical elements for the organism: several of them participate in important functions in the human body, such as enzymatic reactions and redox reactions that are of primary importance in the generation and use of metabolic energy and are essential for biochemical functions compatible with life.

Ninety-nine percent of the human body is made up of the four chemical elements that we know are involved in life: carbon (C), hydrogen (H), oxygen (O), and nitrogen (N), of which oxygen is the most abundant. They are the main constituents of proteins, carbohydrates, and lipids. Until 1950, much attention was paid to these elements and their compounds, and relatively little to structures with other elements. It is currently clear that metals such as Cu, Co, Zn, Na, Fe, Mn, Mg, Ca, Mo, and K are essential for life, and our body must contain them in adequate quantities.

An adequate tissue level of trace elements is associated with good health, and the excess or deficiency of them has been correlated with the development of different diseases, including autoimmune, metabolic, neurodegenerative, muscular, and cancer, among others (Wintergerst, Maggini, and Hornig 2007, 301-323; Kehl-Fie and Skaar 2010, 218-224).

Although some of these elements have been known since ancient times, their extraordinary biological importance was not known, as we have indicated, until the end of the 19th century and the beginning of the 20th century. The research of Gabriel Bertrand, who demonstrated the importance of the mineral components of metalloenzymes, contributed decisively to this. He described their function and proposed calling them something like "small chemicals" and, later, "trace elements." He also worked on other applications related to trace elements in agronomy.

Subsequently, Jacques Ménétrier (1908-1986) confirmed the importance of trace elements in health, finding several of them to be enzymatic cofactors (Deville and Deville 2002).

Trace elements are now recognized as key nutrients for human health and their deficiency leads to the appearance of various dysfunctions and conditions, such as the propensity to viral and bacterial infections, inflammatory processes, and the increased risk of contracting other severe diseases, such as cancer (Zhang 2025).

The function of Trace Elements

Trace Elements fulfill multiple and fundamental functions for life, such as the oxidation and reduction of molecules (redox reactions), or are cofactors of enzymes crucial for existence. Some of them control biological processes, such as the binding of molecules to their receptor sites on cell membranes. They may also be important in inducing the expression of some genes. In some cases, they bind to fundamental sites of proteins from which they acquire the three-dimensional structure necessary to perform their functions. They are also necessary for the functionality of the ECM, the fundamental environment in which the cell

lives (Werner, Aldrich, Becker, Becker, et al. 2016; Cannas, Loi, Serra, Firinu, et al. 2020).

We show in the following list some examples that we consider representative:

- Zinc, Zn, plays a fundamental role in the proper functioning of the immune system. It is a cofactor of more than 200 enzymes and is crucial for the synthesis of proteins, DNA, RNA, carbohydrates, and fats;
- Manganese, Mn, is also a fundamental component of numerous important enzymes (superoxide dismutase, SOD, dependent on Mn) and a regulator of the presence of ions;
- Selenium, Se, is also a component of numerous enzymes, including those that protect the body from free radicals, which is why it is essential in antioxidant defense. It is also a regulator of the activity of the immune system (superoxide dismutases);
- Copper, Cu, is a fundamental component of antioxidant enzymes and, as such, is involved in antioxidant defense;
- Iron, Fe, is part of hemoglobin and is involved in the respiratory chain;
- Cobalt, Co, is the central component of vitamin B12;
- Fluoride, F, accumulates in the bones and gives them greater resistance as well as the teeth, favoring their remineralization and making them stronger and more resistant;
- Iodine, I, is a chemical element essential for the functioning of the thyroid;
- Lithium, Li, helps regulate sleep cycles and improves mood; and
- Magnesium, Mg, is important for giving structure to bones and is beneficial in the treatment of muscle spasms and anxiety.

Trace Elements as immunomodulators

Some trace elements have been ranked as essential for human health in particular, highlighting their implication in immune function (Maggini, Pierre, and Calder 2018, 1-27). It is currently very clear that there are numerous enzymes whose functions require elements such as Zn, Se, Fe, and Mn. This is why nutrient deficiencies result in an impaired immune system, affecting cell-mediated immunity, antibody production, phagocyte function, the complement system, and cytokine synthesis. This, in turn, leads to the worsening of infections (Wintergerst, Maggini, and Hornig 2007, 301-323).

Among the most recognized with these functions are Se and Zn. Numerous *in vitro* and *in vivo* scientific studies have shown that both have a clear importance on the immune system. Their effects are associated with direct actions on immune competent cells. These two elements have also been reported as essential in numerous biological reactions, such as antioxidant compounds. While oxidation reactions are crucial to life, they can also be harmful. Living beings have numerous systems that interfere with this reaction. These are enzymes, coenzymes, and vitamins, whose function is to keep this interference active and regulate it. If antioxidant levels are low or their functions are inhibited, oxidative stress can occur so high that it can fatally damage cells. The trace elements most strongly involved in keeping these systems balanced are crucial by being part of the active center of enzymes or by maintaining the structural and regulatory configuration of hormones and enzymes. They are also crucial for the balance of the immune system. Numerous studies to date confirm that nutritional alterations significantly vary the immune response, a situation that can favor the triggering of different diseases (Siracusa, Tintelnot, Cortesi and Gagliani 2024, 4-10).

Another trace element strongly involved in the regulation of oxidative stress and modulation of immunity is Mn (Erikson and Ascher 2019). Zhang and collaborators are working on Mn-carrying nanoparticles that can participate in tumor immunotherapy: they could act as an adjuvant to regulate the immune microenvironment or activate the host's immune system in tumor immunotherapy (Zhang, Qi and Cai 2023).

The functions in which these trace elements intervene are so varied and complex that a whole range of research is currently opening up on their mechanisms of action, combining nutrigenic and nutrigenomics, in order to optimize the doses and route of administration for both normal and special diets. Advances in genomics and its application in the study of the interaction between Se and other trace elements have allowed a more complete understanding of their importance in health.

Se, Zn, and Mn: Bibliography

A complete bibliographic search clearly shows the positive impacts on immunity and redox balance produced by the three trace elements zinc, Zn, selenium, Se, and manganese, Mn. We review the main scientific evidence that supports their properties.

In our laboratory, we worked with different concentrations of each of the trace elements Zn, Se and Mn, which seemed to us to be the most powerful in terms of their regulatory function of the immune and redox

systems. We observed that the combination of the three effectively causes an interesting effect on these systems, while at the same time causing a powerful inhibition of the growth of malignant cells. From now on, we will call the combination of the three trace elements Zn, Se and Mn the Antioxidant Complex and symbolize it as OLM.

Se antecedents

The history of Se

Understanding the importance of Se in human health has evolved over the 200 years since its discovery in 1817; from being considered toxic, it is now being recognized as a crucial nutrient with a variety of therapeutic uses.

The chemist Berzelius discovered Se in 1817, and in the following years, some organic compounds containing it were synthesized **(**Franke and Tully 1935, 273-279). It is interesting how knowledge regarding its biological activity evolved over time.

It is considered that the first report of selenium toxicity was made by Marco Polo (1254-1323), a Venetian merchant and traveler who observed that his horses showed symptoms of poisoning after having crossed certain areas of China. He ascertained that the horses used on his trips had been poisoned by ingesting high doses of selenium that had accumulated in certain plants native to a region of China through which he had traveled. After crossing these areas, the horses had injured hooves, general decay, and loss of mane.

The determination of the Se content of several plants native to some regions of the United States made it possible to determine that certain species of these vegetables accumulate extremely high levels of Se (up to several thousand parts per million). Animals that fed in these areas presented symptoms of poisoning, such as a lack of balance, loss of appetite, vomiting, and paralysis, and finally death.

In the 1930s, Se was recognized as the powerful toxic substance present in various types of plants that, when ingested by animals that grazed them, caused chronic poisoning. At the time of its discovery, poisoning was attributed to it in areas of soils and plants abundant in Se, particularly in some regions of China.

From the 1930s to the mid-1950s, many researchers attempted to determine the chemical form of Se present in toxic plants, and animal nutritionists tested the effects of various inorganic selenium compounds and some organic compounds administered to animals. That is to say, until the 1950s, this trace element was considered an important toxin.

In 1957, knowledge regarding the biological activity of Se has advanced significantly when scientist Klaus Schwarz reported that it was the essential component of a dietary preparation, he called Factor 3, which prevented severe liver necrosis in rats. Later, the benefits of Se to humans were revealed by studies carried out by Schwarz and Folds, who demonstrated that dietary Se protected rats from liver necrosis (Schwarz and Foltz 1957, 3292-3293). More recent publications go in the same direction **(**Xu, Lu, Wang, and Feng 2022, 922). As selenide, the chemical form, it is widely distributed along with other heavy elements and presents different forms of oxidation. Its industrial uses are extended to different areas: glass industry, paints, plastics, rubber, and, more recently, the pharmaceutical industry.

The history of the applications of this element changed dramatically after the discoveries of Klaus Schwarz (1914-1978, a leading trace element researcher) of selenoproteins in 1957 and the organic selenium species as selenocystin, selenomethionine, and others. That Se is strongly related to human health is being increasingly confirmed over time (Morris and Mohiuddin 2024).

Currently, numerous studies indicate that Se deficiency is associated with an increased risk of contracting various health conditions, including cardiovascular diseases, infertility, cognitive impairment, and degenerative disorders (Shayganfard 2022, 1032-1059; Shreenath, Hashmi, and Dooley 2024). It is such an important micronutrient and the functions in which it intervenes are so complex that a whole range of research is currently opening up regarding its mechanisms of action, combining nutrigenics and nutrigenomics, in order to optimize the doses to be used and the more appropriate route for administration.

Advances in genomics have allowed us to have better knowledge about the implication of Se and other trace elements on human health.

The main source of Se for humans is the intake of foods and nutritional supplements enriched with Se, which, together with proteins, form low-molecular-weight selenoproteins. These proteins have one or more Se atoms in the amino acids cystine, cysteine, and methionine, forming selenocystin, selenocysteine, selenomethionine, and others. Currently, these organic Se species are the target of numerous investigations since they present antioxidant and anticancer properties.

In recent years, the importance of Se in human health has been made more than clear (Rayman 2012, 1256-1268).

Se deficiency is increasingly recognized as a problem since insufficient intake is correlated with diseases that are preventable with an appropriate diet. Se is currently used for the prevention and/or treatment of diseases

that include a spectrum as varied as cardiovascular diseases, osteoarthritis, rheumatoid arthritis, hypothyroidism, atherosclerosis, cancer, neuronal diseases, and others. Historically, Se deficiency was associated with a type of cardiomyopathy known as Keshan disease, which was initially documented in China in the 1930s. Giving Se supplements to people residing in areas where Keshan disease is endemic resulted in a significant reduction in the incidence of this condition, underscoring the therapeutic potential of Se. Inflammatory bowel disease is another syndrome associated with low Se content in patients who suffer from it.

Several studies have investigated the potential role of Se in cancer prevention and treatment. Selenoproteins are enzymes known to protect against the damage caused by reactive oxygen species (ROS) or free radicals, which are the fuel of numerous processes, such as aging, inflammation, and cancer among others. Se, via selenoproteins, plays an important role in many of the cellular activities that underlie metastasis, including cell adhesion, ECM, degradation and migration, invasion into the blood, and extravasation into secondary tissues and angiogenesis. One of the many related investigations demonstrated that methylselenol, generated from selenomethionine, induces cell proliferation, adhesion, and integrin expression in B16F10 murine melanoma cells, which are metastatic to the lungs of syngeneic C57BL/6J mice (Marciel and Hoffmann 2017, 85-108).

Se and the immune system

Se exhibits a wide range of properties in different live organisms. Several mechanisms have been suggested as mediators of the biological effects of this trace element. Among the most important are the modulation of the immune system, the action as a cofactor of enzymes involved in antioxidant systems, the synthesis and stability of metabolites that act as intermediates of the expression of selenoproteins and selenocompounds, the regulation of kinases, the epigenetic action on genes, ECM modulation, and others (Maggini, Pierre, and Calder 2018, 1531-1558).

Selenium deficiency is accompanied by a significant loss of immunocompetent cells in the spleen and in the lymph nodes (Kiremidjian-Schumacher, Roy, Wishe, Cohen, et al. 1994, 115-27).

On the other hand, Se supplementation, even in individuals with normal levels of the trace element, has a marked immunostimulant effect that includes an increased T lymphocyte proliferation. Lymphocytes from supplemented individuals show an increased response to antigenic stimulation, an ability to develop cytotoxic T lymphocytes, and increased natural killer (NK) cell activity. These effects seem to be closely linked to the ability of Se to increase the expression of receptors for the main

cytokine regulating lymphocyte growth, interleukin 2, IL-2, present on the surface of activated lymphocytes and NK cells (Liu, Chen, Qi, Ma, et al. 2019, 183-188). Animal studies have demonstrated that Se provides anticarcinogenic and antimutagenic effects against Afaltoxin B1 (AFB1). Furthermore, the effects of Se against immunosuppression caused by AFB1 have also been reported (He, Fang, Peng, Cui, et al. 2014, 167-173).

Furthermore, T cells show an upregulated selenophosphate synthetase activity that is directed toward the synthesis of selenocysteine, an essential building block of selenoproteins, so fundamental for the function of activated T cells and for the control of the immune response. It has been shown, *in vitro*, that Se can modulate Treg percentages, promoting optimal immune responses and, at the same time, the expression of specific suppressor molecules.

Also, it has been demonstrated that Se deficiency can compromise cutaneous immunity.

Even in conditions of an impaired immune system, whether due to age or illness, Se is capable of restoring the reactivity of lymphocytes. It has been detected in elderly patients that they recover faster from infections, such as the flu, if they are medicated with nutraceutical products.

On the other hand, it has been shown in patients with a serious risk of sepsis, such as those with multiple trauma or severe burns, that parenteral Se supplementation normalized the values of glutathione, interleukins, and the markers of oxidative status.

In individuals exposed to various parasitic diseases in rural areas of underdeveloped countries, adequate supplementation with trace elements, particularly with Se, achieved effective immunity against parasites.

Regarding viral conditions, various statistical studies have revealed a higher mortality of patients carrying the HIV virus in geographical areas with Se deficiency in the soil. Accordingly, sodium selenite has been effective in patients with severe and rare viral diseases; for example, in cases of hemorrhagic fever in Henan province, China, a reduction in mortality from 100% to 36.6% was achieved.

Selenium deficiency can lead to changes in the viral phenotype, leading to an increase in its virulence through point mutations in the viral genome. In this sense, an increase in the virulence of the Coxsackie B3 virus that causes myocarditis in mice has been demonstrated in infected and Se-deficient animals.

The benefits of Se use have been demonstrated in respiratory infections, predominantly in bacterial and viral pneumonias. It has even been studied extensively in the context of the SARS-CoV-2 pandemic: In mice infected with a coronavirus similar to the one that caused the

COVID-19 tpandemic, Se supplementation was strongly beneficial. A very brief summary of the extensive bibliographic information before and after said pandemic shows the positive impacts on the improvement in immunity produced by certain dietary supplements. Several combinations of trace elements and vitamins have been shown to exert beneficial effects on the antiviral immune system, although in most of these combinations, the contribution of vitamins did not appear to be as effective as that of trace elements, especially Se and Zn.

In studies of various viruses, such as the West Nile virus, the torque teno virus, and others, a high dose of supplements improved the immunity of patients. It has even been proposed to use Se as a preventative against SARS-CoV-2 infection and other viruses (Kieliszek 2023, 655-662).

Se and Zn deficiency is common in children in developing countries due to the high incidence of malnutrition. This causes an increase in the risk of mortality and morbidity due to different conditions, among others, respiratory diseases. The administration of both trace elements shows a decrease in the incidence of respiratory infections.

Se as an antioxidant

As a constituent of selenoproteins, Se actively participates in the integrity of membranes and in the protection of oxidative damage to DNA.

The antioxidant nature of Se differs from that of the other antioxidant nutrients in that it does not act directly but through the molecules that are formed (Burk 2002, 75-79). Glutathione peroxidase (GPx) eliminates hydrogen peroxide and lipid hydroperoxides, thus discarding molecules toxic to the body at the expense of reducing glutathione. Thioredoxin reductases have a large number of biochemical functions, including the redox modulation of thiols, gene transcription, and growth regulation. These proteins have antioxidant functions. Other selenoproteins are responsible for activating and deactivating the thyroid hormone. They are essential for the regulation of T3 levels and thyroid function.

GPx is one of the enzymes that participate in the transformations of reactive oxygen species, catalyzing the reduction of peroxide or lipoperoxide using reduced glutathione as a reductant. This enzyme plays an important role in antioxidant defense due to its location in all organs and tissues as part of the glutathione antioxidant system, which is involved in the pathophysiology of several diseases. There are many GPxs with different cellular locations. In this diverse GPxs, the structure of the catalytic center is preserved almost intact, which reinforces the hypothesis that the mechanism of action for the different forms is the same. Catalase (CAT), like GPx, is responsible for eliminating H2O2 and its cellular

location is similar, but its regulatory mechanisms are different. GPx and glutathione reductase are part of one antioxidant system, and CAT is part of another.

Certain tumor cells resistant to therapy show elevated GPx activity. Some cytokines, such as tumor necrosis factor (TNF-alpha), interferon gamma (IFN-gamma), and interleukin-1 (IL-1), are capable of inhibiting GPx activity.

Se and apoptosis

In addition, this trace element induces apoptosis and inhibits cell proliferation, some of the mechanisms through which the preventive effects of Se against different types of cancer can also be explained. The role of selenoproteins has also been investigated for apoptosis. In epidemiological and clinical studies, it has been proven that Se supplementation in people with initially low Se levels significantly reduced the incidence of lung cancer such as non-small cell lung cancer (NSCLC). This action is attributed to an Se-containing mediator protein, selenium-binding protein 1 (SELENBP1). The level of this protein was low in many human cancer tissues, including NSCLC. The molecular mechanisms of SELENBP1 were investigated by Western blotting or immunofluorescence assay, and a series of in vitro experiments found that an overexpression of SELENBP1 inhibited the proliferation, migration, and invasion of NSCLC cells and induced cell apoptosis. In NSCLC cells, its overexpression also inhibited growth and induced apoptosis.

Se and epigenetics

Regarding epigenetics, even if the genomic DNA sequences are not altered (mitotically stable chromatin), there are mechanisms that modulate gene expression. These mechanisms include DNA modifications, basically the methylation of cytosine to 5-methylcytosine and its oxidation products, and histones (acetylation, methylation, and many others). These molecules interfere with chromosomal packaging and the binding of transactive factors. Recent advances in DNA and RNA sequencing technology have made it possible to study epigenetic marks at the level of the entire genome, thus providing information on the so-called epigenome.

This study is the basis for understanding the changes in the epigenome in each pathology. It is currently known that epigenomes show plasticity throughout the life of an organism, during cell differentiation and in response to multiple external stimuli. Changes in the epigenome are also associated with the onset and progression of cancer and other complex

diseases, such as autoimmune diseases, inflammatory bowel diseases, type 2 diabetes, and cardiovascular diseases. The causality of most of these associations remains unknown, but considering the primary alterability of epigenetic marks, in contrast to the largely stable configuration of a cell's DNA, targeting the epigenome may provide a promising strategy in disease therapy and prevention.

The main determinants of the risk and progression of the above-mentioned common diseases are, in addition to genetic predispositions, environmental factors such as lifestyle and diet. Specific essential trace elements, such as selenium/manganese and zinc, some dietary patterns, and secondary plant compounds have been found to alter epigenetic marks, and evidence is increasing that the modulation of health outcomes by food components is (at least in part) mediated by their epigenetic effects. While macronutrients (e.g., employed as experimental high fat, high protein, or calorie restricted diets) have also been shown to modify epigenetic marks, the mechanistic explanations for these observations are difficult to derive due to the multiple confounding effects elicited by macronutrients and due to composition variability. Therefore, the majority of studies have assessed epigenetic effects in response to intervention with specific micronutrients and secondary plant compounds. In this regard, Se is a particularly interesting micronutrient or oligoelement. Selenium has been found to modify epigenetic marks in studies employing cell systems and animals, and in a limited number of human studies, too. The importance of Se for maintaining optimal health is based on the biological functions exerted by two groups of Se species: members of the selenoprotein family, which are encoded by 25 genes in humans and contain co-translationally inserted selenocysteine, and a non-selenoprotein pool of low-molecular-weight selenium compounds contained in the diet or derived from Se metabolism.

Se and cancer

Selenium has a long way to go in its relationship with cancer. Different aspects of Se in reference to this disease have attracted the attention of oncologists: the radioprotection of healthy tissues, the radiosensitization of malignant cells, its antiedematous effects, its preventive effects on carcinogenesis, among others. Interestingly, these effects were observed in animal models and in human patients (Micke, Schomburg, Buentzel, Kisters, et al. 2009, 3975-3988). In different experimental models, the effect of Se, Zn, K and other trace elements on angiogenesis and carcinogenesis has been studied for decades (Jacobs, Matney, and Griffin 1977, 319-322; Jacobs 1983, 1646-1649).

Current researchers have returned to old reports that showed Se as a molecule with antitumor activity. As we have already pointed out, Waserman's pioneering works demonstrated early, in 1911, the remission of tumors in mice treated with intratumoral Se (Wassermann, Keysser, and Wassermann 1911, 2389-2391). A little later, the regression of subcutaneous tumors was reported after the application of an oral dose of sodium selenite (1 mg/day) (Walker and Klein 1915, 628-629). The antitumor activity can be attributed to different mechanisms, but with a focus on the recognized antioxidant activity of Se. This activity counteracts the action of ROS and prevents their harmful action on different cellular structures, especially on DNA. In 1920, Watson-Williams published a study in which encouraging results were reported of cancer patients treated with selenium colloidal (Watson-Williams 1920, 50-58).

Thus, a wide list of Se compounds in different chemical forms have been studied not only as anticancerous but also as chemopreventives. The preventive action of Se in carcinogenesis via chemical carcinogen has been attributed to its inhibitory effects on DNA methylation, affecting the methylation of certain suppressor genes, which enables their suppressive expression. Epigenetic modifications can perform a significant role in disease occurrence and pathogenesis. DNA methylation and chromatin remodeling are common epigenetic mechanisms of action of Se.

Recent studies demonstrated a statistically significant association of mortality when subgroups of kidney cancer patients with low levels of Se in their blood were compared with those who had normal or high levels of Se. This was not demonstrated for Zn. In other words, Se levels continue to be an important attraction for clinical trials aimed at improving the survival of patients with kidney cancer. The identification of cancer risk biomarkers and their prognosis is currently of crucial importance to obtain the best results for patients.

In the last decades, Se has also been included as a possible radioprotector in healthy tissues. It has been experimentally shown that when administered to mice together with vitamin E, it exerts a protective effect on intestinal irradiation. For example, in irradiated mice, it prevented mucositis (the inflammation of the mucous membranes of the lining of the gastrointestinal tract; this is usually an adverse effect of cancer chemotherapy and radiotherapy treatments).

Mucositis and diarrhea are associated with radiation treatment in patients with uterine cancer or prostate cancer. It is known that in osteosarcoma, the toxicity of cisplatin and the consequent appearance of serious side effects limit its use in the clinic. The joint administration of Se and cispaltine in the form of nanoparticles has allowed a synergistic

combination with excellent results both in vivo and in vitro. As we have already mentioned, an Se compound with antitumor action is being tested in a poorly differentiated thyroid cancer model.

Finally, Se supplementation in patients receiving chemotherapy or radiotherapy has been reported to be beneficial in various types of cancer (Mutlu-Türkoglu, Erbil, Öztezcan, Olgac, et al. 2000, 1905-1913). Supplements with selenium are frequently used by cancer patients with good results (Dennert and Horneber, 2006).

Actually, there are several studies in progress that test the ability of Se to prevent cancer and improve the quality of life of patients receiving chemotherapy, radiotherapy, or other types of stress. If Se is eventually proven to be an effective chemopreventive agent at these levels, a means other than food will be needed to administer it.

The Biosyn Arzneimittel GmbH Laboratory has approved the product *Selenase* based on sodium selenite in the form of an oral solution or tablets. The medical indications for which the use of this product is specifically recommended are numerous; for example, gastrointestinal diseases (malabsorption), hemodialysis (increased loss of Se), high demand of Se due to chronic diseases (malignant tumors and others), increase in radicals and peroxides (inflammation, infection), external causes (such as chemotherapy and radiotherapy), moments of stress and special physical-psychological effort, elderly patients, pregnancy, breastfeeding, and in the case of diets such as vegetarian, vegan, etc.

All this background and our results obtained in other experimental models seem sufficient to include this trace element as part of the combination that we call Antioxidant Complex and symbolize as OLM.

Zn antecedents

The history of Zn

Zinc, Zn, is an element known to humans since ancient times; it was used by the Greeks and Romans in the Bronze Age, at least 3000 years before the birth of Christ. Brass, which is an alloy of copper and zinc, has been used since then.

During the second half of the 18th century, the development of chemistry reached great importance. At the end of that century, many chemical elements were discovered, such as manganese (Mn), molybdenum (Mo), Uranium (Ur), Hydrogen (H), and others.

In 1746, Andreas Sigismund Marggraf, a German chemist, pioneered analytical chemistry in Berlin, isolated zinc, and identified it for the first

time as a new metal from carbon. Its name comes from the German *zink*, which seems to derive from the Persian *sing*, which means stone. From a biological point of view, it is involved in numerous life processes and is an essential trace element for human health.

Despite being used since ancient times, its essential status was only established in 1930, based on the results obtained in experiments carried out on animals that demonstrated the importance of Zn in animal growth (Todd, Elvehjem, and Hart 1933, 146–156). Only decades later was its prominence in human health recognized. It is currently known that it is an essential nutrient for life and is part of the active center of hundreds of enzymes. There are numerous vital —catalytic, structural, and regulatory — functions in which it participates. It is present in all organs, tissues, fluids, and secretions of the human body. Epidermal, gastrointestinal, central nervous, immune, skeletal, and reproductive systems are the organs most affected clinically by zinc deficiency (Roohani, Hurrell, Kelishadi, and Schulin 2013, 144-157).

The most important biological functions of Zn include the maintenance of normal tissues, care of the immune system, participation in DNA repair, protein synthesis, control of the redox system by being part of antioxidant enzymes, cell replication, and tissue repair (Lee, Bang, Lee, Lee et al. 2019, 316-325). The importance of Zn was established from experiments carried out in 1930 on animals and later, in the second half of the last century, it was determined in humans. Its essentiality was established when it was demonstrated that important enzymes required Zn for their activities. We now know that there are more than 200 to 300 enzymes and more than 1000 transcription factors that require it, and the number of functions in which it is recognized to intervene is increasing, such as the synthesis and generation of proteins, DNA, RNA, carbohydrates, and fats as well as for the synthesis of hormones, including thyroid hormones. Its relationship with growth and good health is clearly observed in children to whom it is supplied as a micronutrient, and even in healthy individuals. It is known to play structural roles in metalloproteins such as Cu and Zn-dependent superoxide dismutase (SOD-CuZn), where Zn binds to a tetrahedral complex with four cysteines called “Zn fingers.” It is also involved in the biochemical and hormonal regulation of several endocrine systems, such as the secretion of prolactin and insulin.

The human body cannot store zinc reserves, so a deficiency can arise relatively quickly if the diet is improper. Actually, severe zinc deficiency around the world is rare, but mild deficiencies are common in children of rural areas and in the poorest communities, where it constitutes an

important risk factor associated with different diseases (Prasad 2013, 176-190; Maxfield, Shukla, and Crane 2024).

As indicated by the abundant existing literature, it is essential due to its important intervention in the immune response and its antioxidant capacity.

Zn and the immune system

Its great implication in the body's immune response, both innate and adaptive, has been known for decades. In 2005, in vivo experimental studies demonstrated that in mice on a Zn-deficient diet, both B and T lymphocytes had a lower proliferation capacity in response to mitogens than animals fed normal food. In the innate response, its absence negatively affects the recruitment of neutrophils. It is also known that it regulates the activity of peripheral blood mononuclear cells by modulating the release of cytokines, both IL-1 (interleukin-1), IL-6 (interleukin-6), and others such as TNF-α (tumor necrosis factor alpha) and IFN-γ (interferon gamma), depending on the dose used. In peripheral blood mononuclear cells, it was found that the incorporation of Zn as a sulfate at a concentration of 0.5 μM to mononuclear cells extracted from peripheral blood induced an increase in the interleukins IL-6 and TNF-α, with a peak between 16 and 24 hours. Also through experimental studies, the induction of IFN-γ was demonstrated working with peripheral blood monocytes, meaning that the immunostimulating action of Zn is exerted through the production of cytokines. It has also been shown that Zn deficiency influences the size of the thymus, which is significantly reduced in Zn-poor diets. Zn acts on the maturation of T cells through thymulin, or thymic factor, a peptide that requires Zn as a cofactor. Likewise, it induces the proliferation of cytotoxic CD8+ cells. CD4+ cells are also affected by Zn deficiency, producing an imbalance between Th1 and Th2 subpopulations in these cases. The balance between these populations is essential for the proper functioning of the immune system, and for this Zn levels are very important. Supplementation with this trace element reverses this situation.

Likewise, it has been proven that Zn deficiency also affects the immune response mediated by B cells; therefore, the production of antibodies is affected by a lack of this micronutrient. Zn supplementation has also been beneficial in stimulating the immune response of healthy subjects since it increases the activity of NK cells, especially in elderly subjects.

That is, the presence of Zn is essential for the development and maintenance of immune system cells, both innate and adaptive. When Zn homeostasis is altered, the activation and maturation of lymphocytes is deficient, communication through cytokines is also deficient, and the

immune response is weakened. Along these lines, it has been shown that this trace element increases the activity of NK cells, especially in elderly people.

Numerous investigations support the benefits of oral Zn supplementation for different skin conditions; among others, the improvement in the condition of patients with severe burns.

Zn supplementation is beneficial for the immune system even in healthy subjects, especially those at an advanced age. In this case, a drastic increase in the activity of NK cells has been demonstrated (Wintergerst, Maggini, and Hornig 2007, 301-323).

In addition, a favorable response has been observed in the treatment of prolonged or severe diarrhea and acute respiratory infections, predominantly pneumonia. The administration of Zn together with Se accelerates the recovery of children affected with severe pneumonia. The administration of the trace element as a micronutrient shows a decrease in the incidence of respiratory infections, including pneumonia, in that population group. The same is observed in other viruses, such as that caused by the Nile virus, which is why it was used during the pandemic caused by the SARS-COV-2 virus with the aim of strengthening the immune system of patients affected by this and other respiratory viruses.

It is known that Zn intervenes in the expression of major histocompatibility complex proteins and costimulatory molecules, essential for efficient antigen presentation. In a study carried out on hospitalized patients in a critical condition, the levels of Zn, Se, Mn and Cu were measured. All patients received daily Zn supplementation (0.5 mg/day) through a trace element formulation. It was shown that those in whom plasma Zn was increased significantly presented a better evolution than those in whom it was not.

Zn as an antioxidant

The importance of Zn in the regulation of oxidative stress is clear (Cui, Xu, Lv, and Guo 2023). Zn integrate the active center of many antioxidant enzymes. The tissue levels of antioxidant enzymes decrease over time, and this causes free radicals to react with molecules of biological importance to the body, thus producing a greater risk of developing severe diseases. Zn, as a cofactor of the enzyme SOD, becomes extremely important in maintaining human health (Chu, Jian, and Chu 2025, 609).

An enriched mixture of Zn and Se (called SZMs) in the diet for six weeks was tested by Yan and Chang in laboratory animals, mice, and its antitumor and antioxidant effects evaluated. Antioxidant activity was measured through the lipid peroxide products formed and the activity of antioxidant enzymes. Glutathione peroxidase and superoxide dismutase

showed increased activity. Furthermore, using a lung tumor model, it was seen that SZMs caused a decrease in the number of developed tumors (Yan and Chang 2012, 236-241). The results of this study consolidate the hypothesis of the implication of the two trace elements in both systems: the immune and the oxidative. This conclusion goes in the direction of our initial hypothesis.

Zn and cancer

It has also been widely reported that, based on its immunostimulant and antioxidant properties, Zn is a protector against the development of cancer. Its deficiency in the diet is correlated in some research with the development of various types of cancer. The role of zinc in the cellular and humoral immune response is of particular importance. Zn regulates cyclooxygenase 2 (COX-2), an enzyme related to inflammation and pain and involved in the development of various cancers (Prasad 2013, 176-190; Złowocka-Perłowska, Baszuk, Marciniak, Derkacz, et al. 2024). Due to the multitude of functions performed by Zn, it can be assumed to play a leading role in defending against the initiation and promotion of tumors, although the mechanism of this role is not fully known.

Zn administration also showed beneficial effects in post-radiation therapy patients as well as in patients who received localized radiotherapy. In patients with Hodgkin's lymphoma and Burkitt's lymphoma, serious Se and Zn deficiencies were observed, which is associated with the malignancy of the pathologies. In this case, as well as in other investigations, the authors recommend supplementation with both trace elements associated with standard chemotherapy and radiotherapy.

In conclusion, the influence of zinc on the immune system, transcription factors, cell differentiation and proliferation, DNA and RNA synthesis and repair, enzyme activation or inhibition, the regulation of cellular signaling, and the stabilization of the cell structure and membranes are some of the functions of Zn.

This extensive bibliography and our previous results obtained by employing different experimental models justify the inclusion of Zn in the Antioxidant Complex.

Mn antecedents

The history of Mn

Mn is a trace element currently recognized as essential for life (Erikson and Aschner 2019).

It was discovered in 1771 by Carl Wilhelm Scheele, a Swedish chemist born in what is now German territory, who is known for his pharmaceutical work and for the discovery of many elements and chemical substances, the most important of which was oxygen. He was one of the best chemists of the 18th century. In 1774, Mn was separated from other elements by Johann Gottlieb Gahn, a Swedish chemist and metallurgist. Gahn was able to isolate metallic manganese by reducing the manganese dioxide compound.

At the beginning of the 19th century, Mn began to be tested in steel alloys. In 1816, it was proven that it hardened steel without making it more brittle, and this generated a significant number of industrial applications. Mn is found in nature in the form of carbonates, oxides, and silicates. It is very chemically active, can react with several other elements, and has a great diversity of functions. Its use in health is more important today than ever.

Mitochondria are a major site of ROS production in the cell. ROS are formed as a product of oxygen metabolism. The production of an aberrant amount of ROS can cause dramatic changes in cellular function. Cells have a battery of antioxidant enzymes that can deal with the hyperproduction of ROS in the mitochondria, basically superoxide dismutases such as manganese superoxide dismutase (MnSOD). In this way, the trace element Mn also behaves as an antioxidant different from the classic ones since its action is not direct but is expressed from the enzymes of which it is a part.

Mn is a cofactor of the above-mentioned SOD enzyme and other enzymes such as glycosyltransferases, necessary for the synthesis of the proteoglycans essential for the formation of cartilage and healthy bones. The role of Mn in maintaining critical functions for human health is widely known. Its role as a catalyst in manganese-dependent SOD (MnSOD) has been highlighted, which means this trace element plays an important role in protection against oxidative damage. For example, it has been shown that this enzyme, together with catalase (CAT), plays a decisive role in controlling the damage caused by toxins, such as the herbicide Paraquat, that generate a large production of ROS. Elevated activated SOD has been shown to prevent DNA fragmentation caused by caspases and the loss of mitochondrial transmembrane potential. Elevation of the concentration of ROS is capable of inducing apoptosis and the death of T lymphocytes.

This micronutrient also intervenes in the survival of immunogenic cells since MnSOD is capable of inhibiting the death of these cells by stopping the ROS production mechanisms. In research animals, Mn deficiency influenced the decrease of MnSOD and therefore predisposed the research subjects to the development of different dysfunctions, including skin inflammation. It is also known that the antioxidant activity of MnSOD is

capable of regulating the number of antigen-specific CD8+ cytotoxic T lymphocytes in mice subjected to viral infections.

On the other hand, several investigations demonstrate the relevance of Mn in the protection against carcinogenesis. For example, a deficiency of Mn is associated with the promotion of hepatocarcinogenesis.

Finally, recent research is taking into account the participation of Mn in novel anticancer therapies. Interesting results have been obtained working with nanoparticles and synthetic molecules containing Mn, which demonstrated antitumor and proapoptotic effects (Hou, Tian, Yan, Zhang, et al. 2020, 3927-3940).

This brief summary of the biological activities reported for Mn supports the inclusion of this trace element in the composition of the Antioxidant Complex.

There is abundant scientific information about the importance of Se, Zn, and Mn in human health, as well as of their possible participation in the prevention of certain pathologies or intervention by strengthening the body's ability to recover if the disease has already established itself.

In addition, the trace elements Zn, Se, and Mn are fully involved in the modulation of the extracellular matrix in different pathologies (Crescenti, Paredes, Cuba, Perazzo, et al. 2022).

This background led us to hypothesize that a combination of these three trace elements could increase the individual effect of each one separately, and then together constitute a compound with a very good regulatory capacity of the redox system and the immune system acting through the ECM.

Effects of the Antioxidant Complex on different experimental models. Introductory studies

Based on the bibliographic data on the effects of these three trace elements on human health, we developed the hypothesis that their combination could exert an excellent joint action on both the immune system and the redox system. Therefore, from the beginning of the experiments, we thought that this combination of trace elements would be beneficial for human health and could act through multiple targets: cell proliferation and cell death, cells of the immune system, redox balance, and on ECM. It is well known that ECM regulates tissue development and homeostasis, and that its deregulation contributes to the development of many diseases, including cancer and others in which the immune system is involved.

We proposed to carry out studies in vitro on cell lines of different types, tumorigenic and non-tumorigenic, with the purpose of testing our hypothesis, and *in vivo* on different models on laboratory animals.