Magnesium, calcium and cancer

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Abstract. Magnesium ion (Mg$^{2+}$) and calcium ion (Ca$^{2+}$) control a diverse and important range of cellular processes, such as gene transcription, cell proliferation, neoplastic transformation, immune response and therapeutic treatment. Their characteristic biologic antagonism makes it important to treat the most important aspects of that competitive behavior together. This synopsis aims to be a useful means of promoting further research on the relationship between both cations and human health affected by environmental conditions.

Key words: magnesium, calcium, cancer, magnesium/calcium ratio, carcinogenesis

An exceptional, complete and concise description of the biological properties of magnesium, related to the human health, was published in 1985 [1]. This brief review has for aim to present an evaluation of the most important experimental publications concerning the subject. The extraordinary evolution of the technology, and the consequent creation of ecologic problems, emphasizes the importance of a better knowledge of the interactions between magnesium and calcium, so relevant to biological equilibrium in health and in illness. The purpose of this review is to provide the references from research work performed in different specialized biologic fields where Mg$^{2+}$ and Ca$^{2+}$ have an important involvement.

Magnesium and calcium cellular activity

Magnesium is involved in energy metabolism, in nerve impulse transmission, in muscle contraction, and in bone mineralization. It forms complexes with ATP, ADP and GTP, being necessary for the activity of enzymes implicated in the transfer of phosphate groups such as gluco-kinase, phosphofructo-kinase, phosphoglycerate-kinase, pyruvate-kinase, DNA-polymerase, ribonucleases, adenylycyclase, phosphodiesterases, guanilate-cyclase, ATPases and GTPases [2]. It is intercalated, by electrostatic bonds, between two oxygen atoms, each one carrying a negative charge resulting from its ionization.

The compartmentalization of Mg$^{2+}$ within the cell is a key element in coordinated control by regulation of pathways in which the rate-limiting steps are transphosphorylation reactions [3]. Mg$^{2+}$ has a role in the regulation of protein synthesis and protein synthesis is very sensitive to small changes of intracellular Mg$^{2+}$ within physiological ranges and the onset of DNA synthesis is dependent on the rate of protein synthesis [4-7]. Both Mg$^{2+}$ and Ca$^{2+}$ are implicated in rather similar biochemical processes that regulate the metabolism and the reproduction of the eukaryotic cell [8]. Mg$^{2+}$ binds to sites of fundamental importance to chromosome structure and is implicated in its metabolism, because it is a necessary cofactor for DNA synthesis and without it DNA synthesis is hindered and cellular division is stopped [9].

The cells have a system of signals, channels, and a large family of Ca$^{2+}$-binding effector proteins which produce and relay the Ca$^{2+}$-signals that start and coordinate a vast array of cellular functions and even gene transformations. Cell-cycle transit and differentiation can change dramatically along the different stages of carcinogenesis. Depending upon the physiological environment, there are cases in which the roles of Mg$^{2+}$ and Ca$^{2+}$ are antagonistic to each other. Calcium antagonists and their mode of action were described in 1986 [10]. Mg$^{2+}$ effects on the cell plasma membrane are shown by its competition with Ca$^{2+}$ from the CaATP molecule, its activation as MgATP of both the Ca$^{2+}$-pump and the
Na+-pump, and for the exchange of Na+ against K+ because the Na/K ATPase protein needs Mg2+ and PO4"3- ions. In this way, Mg2+ is responsible for the functioning of the transmembrane mechanism that maintains the gradients of Na+ and K+ [11]. At physiological levels Mg2+ is not genotoxic, but it is highly required to keep genomic stability [12].

**Magnesium and calcium in carcinogenesis**

Carcinogenesis is a multistage and a multifactor-implicated process in which Mg2+ plays an important role. Besides being involved in biochemical reactions that are crucial to cell proliferation, angiogenesis and apoptosis, another of its important effects is its influence on the biological responses of immuno-inflammatory cells. A first step of carcinogenesis is the interaction of the carcinogenic factor with the plasma membrane of the cell, followed by the essential condition of oncogene-induction, further evolution of which leads to the neoplasia. There are different types of carcinogenic agents: organic chemicals, radiation, viruses and heredity factors. The oncogene is the result of a process of changing the DNA in some of the cells, and these cells, by a subsequent action of promoters that enhance the formation and development of abnormal cells, result in the formation of a malignant transformation (tumor). The oncogene derives from a protooncogene that encode proteins of regulation of cell growth and differentiation. The protooncogene to oncogene transformation occurs by a gene mutation or an increased expression level.

The characteristics of the plasma membrane are changed to initiate the carcinogenesis process. There is a drastic change in ionic flux from the outer and inner cell membranes in the impaired membranes of cancer, as is also observed in magnesium deficiency. The transport of ions is dependent on both the concentration gradient of the ion and the electric potential across the membrane, which is affected by the membrane surface charge [13, 14]. The thioacetamide intoxication and hepatocarcinogenesis produced by its prolonged feeding to animals have appeared as a progressive phenomenon in which morphological changes were associated with important biochemical modifications. Changes in the permeability of plasma cell membrane induce an indiscriminate in- and outflux of extra- and intracellular cations, and produce an increased Ca2+ intracellular accumulation with a consequent injury of the mitochondria by Ca2+-overload. The cell membrane permeability change appears to be produced by modifications in the phospholipid metabolism, as shown by increased incorporation of radioactive phosphorus into the acidic phospholipids: phosphatidyl ethanolamine and phosphatidyl serine [15]. Compared with control animals, the affected tissues, the hepatoma and the cholangiocarcinoma induced by thioacetamide, the hepatoma and the cholangiocarcinoma induced by 4-dimethylaminoazobenzene, presented increased Ca2+ and Na+, whereas Mg2+ and K+ were decreased [16]. A study with eight different types of transplanted tumors in animals showed that the Mg/Ca ratio increases sharply with the tumor growth and after reaching a maximum, it decreases as the tumor grows further. The increase is sharp in fast growing tumors and more gradual in slow growing ones. The higher growth rate with an increase in Mg2+ concentration was considered as an implication of Mg2+ in the high metabolic activity of the tumor [17]. Concerning tumor growth, Mg2+ deficiency has been reported to inhibit primary tumor growth but to favor metastasis in mice; this is a paradoxical behavior that depends on Mg2+ status [18, 19].

**Calcium signalling, magnesium-calcium antagonism and magnesium to calcium ratio**

Calcium ions play a significant role in a great variety of cellular activities acting as a second messenger in Ca2+-signalling. The signalling system is based on the generation of brief pulses of Ca2+ resulting from the coordinated release of Ca2+ from internal stores using either inositol 1,4,5-triphosphate or ryanodine receptors [20], and different types of cells using components selected from an array of signalling homeostatic and sensory mechanisms [21]. The effects of Mg2+ are generally opposite to those of Ca2+, it has a Ca2+ antagonist effect, shown by inhibition of Ca2+ receptor- and voltage dependent channels [22]. In this way it protects mitochondria against calcium overload [23], an effect that appears related to the mitochondrial permeability transition pore, and to the mitochondrial transmembrane potential.

The ratio of magnesium to calcium is very important in relation to the causes and prevention of a number of disorders, such as myocardial infarction or arrhythmia, atherosclerosis, hypertension, urolithiasis, and infant-death syndrome. A higher Mg/Ca ratio is desirable because of the beneficial role of Mg2+. The same type of Mg/Ca ratio is required in the case of carcinogenesis which is a multistage and
multifactor process, in which Mg$^{2+}$ plays an important role that is the well known effects of Mg$^{2+}$ deficiency and supplementary therapy.

Extracellular Ca$^{2+}$ influx, induced by trivalent metal ions (Fe$^{3+}$, Al$^{3+}$, In$^{3+}$, La$^{3+}$ and Ga$^{3+}$), is enhanced by the ATP ligand that inhibits the polymerization of the solvated cation taking place at physiological pH. This consequently permits its biological activity; thus the lack of correlation between lipid peroxidation and increased Ca$^{2+}$ uptake has been demonstrated [24]. This Ca$^{2+}$ accumulation is not specific for tumors and also takes place in other pathological states such as experimental calciphylaxis, tissue inflammation and infectious focus, where the metabolism of both Mg$^{2+}$ and Ca$^{2+}$ is locally modified. This interpretation of the phenomenon is also valid for the cations already mentioned which are implicated in isomorphous ionic replacement [25]. The La$^{3+}$ acts in a different way to that of a calcium antagonist because it inhibits the calcium-ATPase cell pump, by stopping its protein in a phosphorylated form and thus blocking the transition from the first form of the protein to the second form, which performs ATP hydrolysis [26]. Another type of process is shown by Mg$^{2+}$. It influences the CaATPase and the Na/K ATPase functions by shifting the relation of the pump towards exchange. Mg$^{2+}$ competes with Ca$^{2+}$ from the CaATPase, and Mg$^{2+}$ as MgATPase activates both the Ca-pump and the Na-pump [27]. The concentration of Mg$^{2+}$ stimulates the phosphorylation of the Ca-pump protein of the sarcoplasmic reticulum by inorganic phosphate, but this effect is reversed by a high Mg$^{2+}$ concentration. There are two conformational forms of the enzyme: E1 and E2. The former is the form of enzyme that presents two high-affinity Ca$^{2+}$ binding sites, and it is phosphorylated by ATP when Ca$^{2+}$ is bound. Mg$^{2+}$ is weakly-bound in the two Ca$^{2+}$ binding sites and to a third site, known to be present on F1, with the result of stabilization of F1 at the expense of F2, particularly when the Mg$^{2+}$ concentration is high. This stabilization of F1, at pH 6.2 and 25°C, is a highly cooperative function of Mg$^{2+}$ concentration and appears not prevented by increased phosphate concentration [28].

An experimental series of studies using Ico OFI (IOPS, Caw) mice, bearing genetically induced lymphoma genes, have permitted to verify the role of Ca$^{2+}$-signalling induced by iron complexed by ATP acting as Ca$^{2+}$ signal activation agent. In comparison with untreated lymphomagene-bearing control mice, the iron and ATP-treated showed a reduction of life span (or survival), as well as a lack of the characteristic accumulation of a substantial volume of ascites. These results are an index of the degree of malignancy of the induced lymphoma and their relationship with the increased intracellular calcium homeostasis changes. Spleen and liver, which are the target organs besides the lymph nodes, showed, with respect to the control animals, a generally lower pattern of Mg$^{2+}$ distribution, whereas the values in solid tumor-bearing animals were very high, as in the tumor [29]. When the treatments were done by percutaneous, subcutaneous and intraperitoneal administration, both ATP compounds induced a generalized lymphoadenitis, which, in the case of FeATP, led to solid lymphomas. The effects in any case were more important in the injection area, apparently due to an immediate interaction of the Ca$^{2+}$ signal with the oncogenes [30]. It is very important to note that in all these experiments the Mg$^{2+}$ concentrations of the animals dead before 9 months were lower than in the control group. This observation indicates a direct relationship between carcinogenesis evolution and Mg$^{2+}$ deficiency.

The implication of Mg$^{2+}$ in normal and pathological cellular physiology is a well known phenomenon [31-38]. Small changes in Mg$^{2+}$ levels may have important effects on cardiac excitability and on vascular tone. Accordingly, Mg$^{2+}$ may be important in the regulation of blood pressure, and perturbations in cellular Mg$^{2+}$ homeostasis could play a role in pathophysiological processes underlying blood pressure elevation [39]. Altered cellular physiology occurs after acute exposure to severe Mg$^{2+}$ deficiency, whereas long-term exposure to a moderate deficiency was found to accelerate cellular senescence. These observations suggest that chronic Mg$^{2+}$ deficiency may be a promoter of age-related diseases [40]. A study on common iliac arteries with aging demonstrated that the magnesium to calcium ratios were higher at an early stage of the accumulation of calcium and phosphorus in the arteries than at an advanced stage of the accumulation [41]. Moderate dietary Mg$^{2+}$ deprivation results in calcium retention and altered potassium and phosphorus excretion [42]. A high Mg$^{2+}$ consumption is linked to a significantly lower risk of colorectal adenoma, particularly for subjects with a high Mg$^{2+}$:Ca$^{2+}$ intake [43]. The increased calcium/magnesium ratio in cells from hypertensive animals is a pathogenetic factor for the development of atherosclerosis and hypertension [44]. Extracellular Mg$^{2+}$ regulates nuclear and perinuclear free Ca$^{2+}$ in cerebral vascular smooth muscle cells that...
indicates the possible implication of hypomagnesemia on cerebral-central nervous system pathobiology, and, particularly on alcohol provoked strokes [45].

**Synergism in carcinogenesis**

Using the animal model of mice bearing genetically induced lymphoma genes, the complex and multifactor-determined evolution of carcinogenesis has been investigated [46]. The evaluation of accumulated experimental results on the action of iron provoking cell injury, indicates that it is rather the consequence of an inhibition of the cytosolic Ca\(^{2+}\) concentration regulatory system than an effect of iron-induced lipid peroxidation on cells. After parenteral iron administration of iron, complemented by determination of the *in vivo* uptake of \(^{59}\)Fe-labeled ferric gluconate and ferric-ATP complex, the results of mortality, clinical and histopathological examination have demonstrated a synergism between radiofrequency and ferric gluconate, and the increased risk of radiofrequency exposure, when it is simultaneous to parenteral iron administration [47]. These results corroborate the importance of the pathological evolution of iron overload to a neoplasia that has been presented as statistical evidence [48], and by the induction of sarcomas at the site of parenteral administration of iron dextran in animals as well as in humans [49, 50]. A similar study of the synergism in the system Al\(^{3+}\)-radiofrequency, showed an early mortality increase in concomitance with lymphoid element proliferations and infiltration of the liver and spleen [51], supporting the concept of Al\(^{3+}\) competition with Mg\(^{2+}\) implicated in the inhibition of the buffering and extruding extracellular Ca\(^{2+}\) system [52]. Mg\(^{2+}\) is one of the cations most affected by Al\(^{3+}\) interference, its function, more than any other cation, is affected by competition with Al\(^{3+}\) [53], and size similarity is a dominant factor over the charge identity concerning metal ion competition [54, 55].

**Gallium in cancer diagnosis and therapy**

The mechanism of accumulation of the widely used diagnostic agent \(^{67}\)Ga-citrate can be interpreted by means of the isomorphous replacement model. The *in vitro* ATPase inhibition by Ga\(^{3+}\) appears to be the result of the ionic replacement of Mg\(^{2+}\) by Ga\(^{3+}\). This replacement may be the main cause of radioactive Ga\(^{3+}\) accumulation by tissues: a similar ionic radius for Mg\(^{2+}\) = 0.65 Å than Ga\(^{3+}\) = 0.62 Å, but a higher valence for Ga\(^{3+}\) makes a competitive binding of Ga\(^{3+}\) by Na,K-Mg\(^{2+}\)-dependent ATPase possible [56]. It is interesting to note that this ionic replacement is also valid for iron-binding sites because the Fe\(^{3+}\) ionic radius is equal to 0.64Å [57]. The factors affecting \(^{67}\)Ga accumulation have been well pointed out in an extended review [58]. Ribonucleotide reductase controls the balance of the deoxyribonucleotide pools, and changes in its activity can alter the mutation of cells [59]. Augmented ribonucleotide activity has been associated with disease states including cancer [60, 61]. Inhibition of DNA synthesis by gallium is probably due to a combination of a block in iron availability to ribonucleotide reductase and a direct inhibition of the enzyme by gallium [62]. This could explain why gallium nitrate has been used in cancer chemotherapy [62, 63].

**Hyperthermic treatment of cancer**

In hyperthermic treatment of experimental tumors submitted *in vivo* to hyperthermia, done in the presence, or not, of La\(^{3+}\), modifications in the concentration of extra- and intracellular cations have been shown. Sacrificed immediately after hyperthermia, only the group treated with La\(^{3+}\) presented ionic changes: lower Mg\(^{2+}\) and K\(^{+}\). After 48 hours, in both cases, Ca\(^{2+}\) and Na\(^{+}\) were increased while Mg\(^{2+}\) and K\(^{+}\) were decreased [64]. These changes in the ionic composition: increased Ca\(^{2+}\) and decreased K\(^{+}\) concentration, and the additional decrease in ATP concentration, appear as an effect of the hyperthermic inhibition of the ATP-dependent extrusion mechanisms of the cell which induces ionic environmental modifications [65].

Differences in membrane fluidity may account for differences of thermal sensitivity. To increase the cytotoxicity of tumor cells at hyperthermia temperatures, a variety of chemotherapeutic agents have been tested. The increase of Ca\(^{2+}\)-uptake by tumor cells during hyperthermia appears linked to a failure of the Ca\(^{3+}\)-pump in relation to an inhibition of Ca-ATPase [66], and this alteration of the ATPase system might be related to changes in ATP concentration during hyperthermia, as demonstrated by \(^{31}\)P-nuclear magnetic resonance spectroscopy [67]. The changes in membrane fluidity maybe implicated when, in infarct from permanent middle cerebral artery occlusion in the rat, Mg\(^{2+}\) (as MgSO\(_4\)) is neuroprotective when combined with mild hypothermia, even when the treatment is delayed by several hours [68]. La\(^{3+}\) potentates hyperthermia [69].
Magnesium and immune response

Mg\(^{2+}\), besides being involved in biochemical reactions that are crucial to cell proliferation, angiogenesis and apoptosis, shows that one of its important effects is its influence on the response of immune-inflammatory cells, as showed by Mg\(^{2+}\) deficiency in cardiovascular diseases that present changes in the leukocyte subpopulations, which are concomitant with the cardiopathic lesion progression [70].

Tumor growth needs suppression and/or evasion of the host immune system, and immune modulation is considered a way to counter and suppress tumor growth [71-73]. Mg\(^{2+}\) is involved in both carcinogenesis and immune response [74] and it is important to the immune system in both non specific and specific immune responses, innate and acquired immune responses [75, 76]. Abnormal Ca\(^{2+}\)-handling induced by low extracellular in vivo Mg\(^{2+}\) may be at the origin of exacerbated inflammatory responses. It has been suggested that the activation of immune cells is an early event in Mg\(^{2+}\) deficiency [77, 80]. Lymphocyte proliferation is triggered by pulses of increased cellular Ca\(^{2+}\) provoked by ATP, an effect that is transient and controlled by the Ca\(^{2+}\)-buffering system of the cell, in which Mg\(^{2+}\) is implicated [81].

The differences between Mg/Ca ratios in: control OFI (Oncine France, Souche 1) mice (a), treated with NaATP (b), and treated with FeATP complex (c) were as follows: in the spleen a = 0.67 < b = 1.29 < c = 1.40, and in the liver, a = 0.55 < c = 0.58 < b = 1.90. These values agree with the structural perturbations by lymphocyte infiltration and pathologic ascites formation and lymphocytic leukemia manifestations observed in both organs [47]. These differences in correlation with the histopathological observations indicate the role of Mg\(^{2+}\) in both activation of the immune response and in the oncogene evolution leading to a neoplastic development.

The importance of balanced Mg\(^{2+}\) homeostasis and the interaction with the immune system is demonstrated. Both in animal models and in human systems, Mg\(^{2+}\) is involved in inflammation, apoptosis and thymocyte gene expression [78]. During Mg\(^{2+}\) deficiency there are altered cell growth, modifications of ion fluxes and oxidative stress. The observation of the induction of genes involved in the protection and repair in cells from Mg\(^{2+}\)-deficient animals is evidence of the role of oxidative stress in the pathobiology of Mg\(^{2+}\) deficiency [79, 80]. On the other hand, several experimental and epidemiological studies have shown an inverse correlation between Mg\(^{2+}\) status and the risk of some cancers, but the relationship between Mg\(^{2+}\) and cancer is complex. In mice, an Mg\(^{2+}\)-deficient diet retards primary tumor growth (up to 70%), but Mg\(^{2+}\)-repletion in these mice increases the primary tumor burden [19].

Radiation, magnesium, calcium and cancer

The mitochondrial production of ATP depends on the nuclear spin and magnetic moment of Mg\(^{2+}\) ion in creatine kinase and ATPase, as a consequence of it, the enzymatic synthesis of ATP is an ion-radical process depending on the external magnetic field and microwave fields that control the spin states of ion-radical pairs and influence the ATP synthesis [81, 82].

Studies have consistently shown an increased risk for childhood leukemia associated with electromagnetic fields [83] and concern is growing about possible pathologic effects on humans due to exposure to electric and magnetic fields [84]. Numerous epidemiological studies have demonstrated an association between cancer and exposure to electromagnetic fields [85]. Not all types of electromagnetic radiation are in fact carcinogens. Low energy waves on the electromagnetic spectrum generally are not. Higher energy radiation, including ultra violet radiation, X rays and gamma radiation, are carcinogens when acting in sufficient doses. Chronic exposure to low doses could be much more harmful than short-term high doses because of lipid peroxidation initiated by free radicals. The cell membranes and cellular organelles are the main targets for free radical attacks. Peroxidation of cell membrane increases with a decreasing dose rate (Petrica effect). Gamma radiation results in damage to the plasma membrane of resting lymphocytes via the generation of high reactive free radical species. This damage is reflected in a rapid increase in plasma membrane permeability and chromosomal instability and the DNA damage induced may be a cause of permanent genetic damage. Ionizing radiation is an activator of oncogenes, and an inactivator of tumor-suppressor genes. Oncogen activation promotes cellular proliferation that is removed by the intervention of tumor suppressors. Under long exposure conditions, radiofrequency (RF) signals at an average SAR of at least 5.0 W/kg are capable of inducing chromosomal damage in human lymphocytes [86]. Mediated Ca\(^{2+}\) signalling processes are involved in electromagnetic field effects on the immune system [87]. An increase in the Ca\(^{2+}\) influx appears to be responsible for the effects of 50 Hz electromagnetic fields on voltage-gated-Ca\(^{2+}\).
channels modulating neuroendocrine cell proliferation and apoptosis [88]. Nanosecond pulsed electric fields, like ligand-mediated responses, release Ca\(^{2+}\) from internal calcium pools and activate plasma membrane Ca\(^{2+}\) influxes through its channels or capacitative Ca\(^{2+}\) entry [89]. The lymphatic system plays a major role in the immune system defending against cancer induced by modulated electromagnetic radiation [90], and assessing occupational exposure risk to microwave radiation [91].

The importance of the effects of RF on the increased Ca\(^{2+}\) signal responsible for the acceleration of carcinogenesis in animals bearing genetically produced lymphogenes is a fundamental argument for the cancer risk from RF exposure [92]. Nanomolar Ca\(^{2+}\) influx induces lymphocyte proliferation and this RF exposure is a possible factor of disruption, by variations in the equilibrium existing between ions, polyanionic macromolecules and glycoproteins of the cell surface, that can be induced by the rhythmic modulation of the RF. Consequently, an extracellular Ca\(^{2+}\)-influx by passive diffusion can produce pulses of Ca\(^{2+}\)-signal which induce lymphoid element proliferation. The effects upon the blood-brain barrier and upon tumor growth in the mammalian brain appear to support this disruption of cell membrane explanation [93]. On the other hand, the absence of increased lipid peroxidation in tissues of RF-treated mice corroborates the independence from oxidative stress [45]. On the other hand, the observed synergism of iron and aluminium, two elements capable of modifying cell Ca\(^{2+}\) homeostasis [46, 50], is another factor implicated in the cancer effects of this type of radiation that corroborates that independence from oxidative stress. This lack of oxidative stress has been demonstrated by other authors [94]. The use of the FeATP complex to evaluate the risk of accelerated carcinogenesis by RF [95] emphasizes the role of the Ca\(^{2+}\) signal influx in the acceleration of oncogenes and the failure of thymus-determined immune defences. In addition to this, the presence of genetically inherited factors or those induced by carcinogens (chemical or physical) is an indispensable condition for the inherent cancer risk of low energy level RF radiation [96].

References


