The Effect of Different Extracellular Magnesium Concentrations on the Growth and Differentiation of Osteosarcoma Cell Line (SaOS-2): *in vitro* Study

By

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Dedication

To my parents for their effort, advice, and endless support who I will be indebted to them my entire life. To my wife, Widad, for her continuous support and love. To my children, Ali and Aurjwan, for being the best son and daughter ever.
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**ABSTRACT**

**Introduction:** Magnesium is the second most abundant intracellular cation and an indispensable cofactor in more than 300 enzymes that are involved in regulation of a variety of cellular functions. It is also an important modulator of the intracellular pH and free calcium concentration, which are crucial for cell motility and proliferation. Therefore, the effects of low and high Mg$^{2+}$ concentrations on the proliferation and differentiation of normal osteoblasts and neoplastic cells have been studied. Moreover, bone growth factors, mainly Bone morphogenetic proteins (BMPs), Transforming growth factor-β (TGF-B), and Platelet-derived growth factor (PDGF) appear to have an essential role in the differentiation and proliferation of osteoblasts and have an important proliferative effect on many types of neoplastic cells. PDGF is expressed by different cells including osteoblasts and also is one of major components of the platelet rich plasma involved in bone fracture healing. It is also known for the promotion of bone formation and remodeling by stimulating proliferation and migration of osteoblasts. Because of the previous work on normal human osteoblastic cells as well as other osteosarcoma cell lines, in this present study, the activity, differentiation, and mineralization ability of the osteosarcoma cell line (SaOS-2) was evaluated in physiological and low Mg$^{2+}$ extracellular concentrations with and without PDGF. The overall goal of our work is to develop better therapeutic approaches for the regulation of abnormal and normal osteoblastic cell function.

**Aim:** To evaluate the growth, differentiation, and mineralization ability of the osteosarcoma cell line (SaOS-2) in physiological and low Mg$^{2+}$ extracellular concentrations with and without PDGF.
**Materials and Methods:** The cell metabolic activity of SaOS-2 cells was evaluated in different Mg$^{2+}$ concentrations (0.01 mM, 0.8 mM) and in Mg-free medium with and without PDGF using the MTT assay. The differentiation and mineralization capabilities of the cells were assessed using an alkaline phosphatase and alizarin red assay, respectively.

**Results:** The absence of extracellular Mg$^{2+}$ had an inhibitory effect on the metabolic activity of SaOS-2 cells. The effect of extracellularly added PDGF on the metabolic activity was not significant despite the Mg$^{2+}$ presence. In addition, the mineralization ability of SaOS-2 cells appeared to be independent of the extracellular Mg$^{2+}$ concentration. The differentiation potential of SaOS-2 cells, assessed by alkaline phosphatase activity, was not significant when cells were incubated for 1 to 3 days. However, when the cells were incubated for 4 or 5 days, the osteoblastic differentiation was dependent on the physiological Mg$^{2+}$ concentration.

**Conclusion:** Metabolic activity and osteoblastic differentiation of SaOS-2 cells are decreased in the absence of extracellular Mg$^{2+}$. However, the presence of extracellular Mg$^{2+}$ does not appear to be essential for the mineralization capability of SaOS-2 cells. The growth factor, PDGF, does not appear to have a significant role in the regulation of the activity of the SaOS-2 cells in the presence or absence of extracellular Mg$^{2+}$. 
Chapter 1: INTRODUCTION

1.1 Osteosarcoma:

Osteosarcoma (OS) is the most common primary malignant bone neoplasm comprising 40-60% of all non-hematopoietic bone malignancies [1], although it is very rare with approximately only 900 cases being diagnosed annually in the United States [2]. It is characterized by a bimodal age distribution and appears to occur more in children, adolescent, and young adults [3] followed by a second peak in the 6th and 7th decades of life. OS is the third most common malignancy disease in children after leukemia and lymphoma [5]. It can exhibit different presentations clinically, as well as on a microscopic basis, which adds to the difficulty in early detection and management. It has been described histologically as high-grade spindled-shape malignant cells that are capable of producing osteoid. Different subtypes have been identified based on the type of the extracellular matrix, tumor grade, and location [4]. The most common affected site is the metaphysis of long bone with distal femur, proximal tibia, and proximal humerus being the most common locations [2]. Metastasis has been reported in approximately 10-20% of the patients with lung being the most common site [2]. The mainstay treatment is surgical resection with adjuvant chemotherapy or/and radiotherapy [2]. The 5-year survival rate with the current treatment regimens reaches approximately 70% and only 20% in patients with metastatic or recurrent disease [2].

Several predisposing factors in OS have been identified such as: Paget disease, radiation exposure, and fibrous dysplasia where other subsets have been linked to be associated with inherited cancer predisposition syndromes such as: Li-Fraumeni syndrome, Bloom syndrome, Hereditary Retinoblastoma, and Rothmund-Thomson syndrome [2].
1.1.1 Osteosarcoma Risk Factors:

A. Genetic risk factors: many inherited cancer predisposition syndromes have been associated with an increased risk of OS. Retinoblastoma gene (Rb1), a tumor suppressor gene, has been strongly associated with OS. It has been estimated that people with mutation of the germ line Rb1 on chromosome 13q14 are 500 times of risk than normal population [4, 5]. Patient with Li-Fraumeni syndrome are harboring a mutation of the gene TP53 and are estimated to carry a risk of 25 times more than normal population [6]. Additional syndromes that are also linked to OS include: Rothmund-Thomson syndrome, Bloom syndrome, and Werner syndrome. All are being autosomal recessive disorders and characterized by mutation in the gene coding for helicase [4].

B. Radiation: In 1920, a case of bone sarcomas developed secondary to radiation for tuberculous arthritis was reported [4].

C. Age of puberty: Earlier onset of puberty has been linked to show a slightly high risk of developing OS [7].

D. Musculoskeletal anomalies: Individuals with musculoskeletal anomalies are at a 6 times higher risk to develop OS [8].

1.1.2 Pathogenesis of Osteosarcoma:

The exact etiology of OS is not well understood, however, there are several factors that are believed to play an important role in the development of the disease. The mutation or deletion of the gene coding the Rb1 or TP53 (both are tumor suppressor genes) results in impairment in their function [9]. This mutation will result mainly secondary to environmental
insults such as UV light or ionizing radiation exposure and cause DNA damages [9]. Another possible factor is linked to growth factors since the 1st peak of OS is always during puberty. Transforming growth factor (TGF) beta protein has been expressed in OS cells and particularly in the high-grade type of the disease and is known to cause alteration in the cell differentiation, cell division, programmed cell death, and bone matrix production [10]. Moreover, Insulin-like growth factors I and II (IGF) have been also found to be overexpressed in the OS cells [11]. OS patients with overexpression of vascular endothelial growth factor (VEGF) have been shown to experience more aggressive behavior of the disease with more propensity to develop metastasis to the lung and subsequently poor prognosis [12]. Notably, many Notch gene pathways such as HEY1, HES1, and NOTCH2 have been connected to upregulation of VEGF [4]. Normal and neoplastic bone matrix both can sequester platelet-derived growth factor (PDGF) and may express it in approximately 86% of the cases [13]. PDGF can be involved in OS progression by affecting the autocrine or paracrine loop [14]. OS cells are known also for the ability to produce PTHrP and interleukin 11(IL-11) which are involved in the initiation of osteoclast activity by stimulating the production of RANKL in osteoblasts [9, 15]. This process results in more progression of the disease by eliciting more bone destruction.

1.1.3 Bone Microenvironment and Osteosarcoma

Different factors such as low oxygen, pH, extracellular calcium level, are involved in mechanisms by which the special bone environment can affect tumor proliferation, invasion, and metabolism. As the tumor grows, areas of decreased blood supply will be evident where hypoxia will develop as a result [17]. The low oxygen tension environment, which is a characteristic environment of bone, is an essential medium for the growth of hypoxia-resistant cancer cells [16].
This environment will cause OS cells to change form aerobic to anaerobic metabolism which will surge the genetic instability of the tumor cells [16]. Another subset of proteins known as the Hypoxia-inducible family (HIF) is also stimulated by the low oxygen tension environment which is then responsible for different cellular functions, such as cell proliferation, angiogenesis, and apoptosis [18, 19]. HIF proteins stimulate the production of the angiogenic factors such as angiopoietin-2, PDGF, and VEGF which are essential for tumor growth [4]. A hypoxic environment may therefore change the OS response to chemotherapy. Adameski et al. [20] found that the chemotherapeutic resistance of OS tumor cells that were cultured primarily in a low oxygen environment was mainly through the HIF 1a independent mechanism. This finding may be clinically relevant when tumor cells may develop resistance to chemotherapy through induction of cancer stem cells proliferation in a low oxygen environment [16].
1.2 Dynamics of Bone

Bone remodeling is a physiologic process which involves bone resorption and formation. It is a very essential process to maintain the integrity of the skeleton and bone repair [21, 22]. Two major components make up the bone tissue: cells and extracellular matrix. It has been estimated that approximately 65% of the matrix is made of inorganic components (mainly calcium and phosphorous) while 35% is organic (mainly type 1 collagen, proteoglycans, and fibronectin). The organic component of the matrix can be further divided into two parts; collagenous and non-collagenous, where the non-collagenous organic part is made of groups of regulatory proteins such as osteocalcin, osteopontin, and bone sialoprotein [21, 22, 23, 25].

The Basic Multicellular Unit (BMU) is an anatomical unit where the cellular events of bone remodeling take place [21]. In the BMU, osteoblasts and osteoclasts play the important role of bone resorption and deposition. The process is fulfilled in four stages [23]:

- Stage I: The activation of the bone surface (bone lining cells)
- Stage II: Bone resorption by stimulating and recruiting osteoclasts
- Stage III: The resting phase between bone resorption and formation
- Stage IV: Bone formation by stimulating and recruiting osteoblasts

Cytokines such as IL-11 and several growth factors play important roles keeping the balance between bone resorption and formation [25]. Parathyroid hormone (PTH) plays a crucial role as well in bone remodeling through a bone homeostasis process. When the level of calcium drops, PTH will be released to stimulate a bone resorption cascade and raise the calcium level back to normal. This continuous process is maintained by release of another hormone, calcitonin. PTH is also known for preventing osteoblast and osteocyte apoptosis [22]. It has been estimated that the resorption process needs around 3 weeks while the deposition will take much longer, 3-4 months.
1.2.1 Cellular Component of Bone Remodeling

Different transcription regulatory factors such as Runx2/Cbfa1 and Wnt have been linked to the differentiation of osteoblasts, the bone forming cells that are responsible for producing the organic bone matrix [22]. Osteoblasts are derived from the multipotent mesenchymal cells. In their active stage, they appear to be lined up on the bone surface. They look very cuboidal and become flat as they move to the inactive stage. As they secrete the matrix, mineralization will take place and they become entrapped in the lacunae. The entrapped cells are called osteocytes, an important cellular component for bone maintenance and homeostasis. Osteocytes, the most abundant bone cell type accounting for 95% of all bone cells, are known to act as mechanosensor and endocrine cells [22]. Osteoblasts are also responsible for the stimulation of osteoclastic cell differentiation through the RANK/RANKL process. Several factors such as macrophage colony stimulating factor (MCSF) are essential for the differentiation of osteoclasts. Osteoclasts, as unique exocrine cells, are bone-resorbing cells derived from macrophages. The bone lining cells, the fourth cell type, have an important role coupling the two processes of bone resorption and bone formation [22].

Bone growth factors are essential for the cell to cell signaling during bone remodeling. They have crucial regulatory and metabolic effects that control the activity of the different bone cell
types [22]. Mainly, they are produced by osteoblasts and incorporated into the extracellular matrix where the constitute less than 1% of the non-collagenous component of the matrix.

Figure 1: the cellular component of bone remodeling [22].
1.2.2 **Bone Growth Factors**

As previously mentioned, growth factors (GFs) are essential for the process of bone remodeling by regulation of osteoblast and osteoclast metabolism. GFs stimulate the proliferation of bone remodeling cells (i.e. osteoblasts) and the release of proteins by binding on the cell membrane receptors. This metabolic process is known as a paracrine effect. Another similar effect of releasing GFs by osteoblasts themselves to increase an initiated metabolic activity is known as an autocrine effect [26].

Several growth factors are essential for bone remodeling:

1. **Platelet-derived growth factor (PDGF)**

PDGF is found in three major subtypes (PDGF-BB, PDGF-AA, PDGF-AB) with each having important roles in cell proliferation and matrix protein synthesis.

2. **Fibroblast-like growth factor (FGF)**

FGF has mitogenic action and is secreted mainly by osteoblasts.

3. **Insulin-like growth factor (IGF)**

There are two types of IGF: (type 1 and type 2) with both having an important role in osteoblast proliferation.

4. **Bone morphogenic proteins (BMPs)**

BMPs are important for the differentiation of mesenchymal stem cells into osteoblasts.

5. **Transforming growth factor-β (TGF-β)**

It is the most important factor in regulating bone metabolism. A large amount of TGF-β stored in bone cells and platelets. For the sake of our experimental work, we will focus on the role of PDGF on bone remodeling and homeostasis.
1.2.2.1 Platelet-Derived Growth factor (PDGF)

PDGF plays an important role in osteoblastic cell chemotaxis and proliferation. It has been found very abundantly in the serum where it is present in alpha granules of platelets and represents the major component of platelet rich plasma [21, 26, 27]. The entire PDGF family has been shown to be composed of four members (A, B, C, and D) which can form disulfide-bonded dimers resulting in five different isomers (PDGF- AA, BB, CC, DD, and AB). Two types of PDGF cellular receptors that mediate its action are found; alpha and beta. In a range of 100ng/ml, PDGF has been show to induce chemotaxis in osteoblasts which in return stimulates osteoblastic cell proliferation and increases protein synthesis [27]. In the normal remodeling and healing process, PDGF promotes the migration, attachment, and proliferation of osteoblasts. PDGF works also with TGF-β1 to provide the optimal osteoblasts chemotaxis and with BMP-2 to stimulate osteoblast differentiation [27, 28].

Figure 2: transcellular signaling in bone remodeling process [24].
1.3 Magnesium ion

Magnesium (Mg$^{2+}$) is the second most abundant intracellular cation after potassium with a concentration of 17 to 20 mM [29]. It is distributed in fairly equal amounts within the endoplasmic reticulum, mitochondria, and the nucleus. Mg$^{2+}$ plays an important role in body physiology acting as a co-factor in more than 300 enzymes [29]. Approximately 15 to 20% of the total body Mg$^{2+}$ is found free with a concentration range of 0.8 mM-1.2 mM. In the absence of the hormonal stimuli, Mg$^{2+}$ has a relatively slow turnover across the cell membranes and is mainly present in a large amount in the cytosol. It usually binds to ATP which helps to keep its concentration between 0.5-1 mM in the cytosol and between 1.2-1.4 mM in the plasma and extracellular fluid. ATP also has to bind to Mg$^{2+}$ in order to become biologically active [29].

Mg$^{2+}$ is crucial in several major cellular processes such as DNA transcription and protein synthesis. It also plays an essential regulatory role in bone dynamics, muscle contractility, and neuromuscular conduction [29]. Furthermore, Mg$^{2+}$ stabilizes the tertiary structure of DNA and RNA which enhances their resistance to oxidative stress [29].

1.3.1 Mg$^{2+}$ Extrusion and Intracellular Action

The process of extrusion of Mg$^{2+}$ from cells is achieved with help from different hormones such as catecholamine and glucagon [31, 33]. Once Mg$^{2+}$ is imported into the circulation or the extracellular matrix it regulates several enzymes such as phosphoinositide which plays an important role in cell signaling. Mg$^{2+}$ also plays a crucial role in the cell cycle. The regulation takes place through Mg$^{2+}$ ATP which controls protein synthesis. The Mg$^{2+}$ ATP level is determined by the MAPKs and the p27 signaling mechanisms. In the extracellular matrix, Mg$^{2+}$ controls
integrin signaling [30, 32, 33]. Mg²⁺ homeostasis is mainly regulated by three organs: the intestines for absorption, kidneys for excretion, and bone for storage [33].

1.3.2 Mg²⁺ Absorption, Transportation, Storage, and Extraction (Mg²⁺ Homeostasis)

Approximately 30-50% of the dietary Mg²⁺ is absorbed where it has been estimated by the US Food and Nutrition Board that the daily amount of Mg²⁺ intake is about 320 mg for men and 420 mg for women. The absorption process is usually compensating for the low Mg²⁺ intake by increasing the amount of absorption up to 80% [30, 33]. Mg²⁺ absorption is done via two mechanisms: paracellular passive transport, and transcellular pathway. The former is being the main pathway that is used in the intestine which accounts for approximately 80-90% of total Mg²⁺ reabsorption [29]. It represents the passive transport of Mg²⁺ between the junctions of the epithelial cells. Claudins 16 and 19 are the most common prime channels. These channels facilitate the reabsorption of a large amount of Mg²⁺ [29]. On the other hand, the transcellular actions involve the active transport of Mg²⁺ across the plasma membrane of the epithelial cells. It mainly occurs in the cecum and colon via apical Mg²⁺ channels transient receptor potential melastatin 6 (TRPM6) and TRPM 7 [29]. This requires an efflux pump (i.e. P-glycoprotein) and also utilizing energy [30, 32]. On the cortical surface and in a form of hydroxyapatite crystals, around 60% of the total body Mg²⁺ is stored in the bone. Mg²⁺ plays an important role marinating the density and strength of the bone. A small amount of Mg²⁺ is stored in the muscle fibers where it is essential in the process of muscle contraction. As with many other minerals, serum Mg²⁺ level is maintained with an equilibrium of the bone resorption (releasing Mg²⁺) and uptake (storing Mg²⁺) [34]. In contrast to the high amount of Mg²⁺ being filtered in the kidney (≈ 2400 mg) only 100 mg is excreted in the
urine. Most of the reabsorption is taken place in the thick ascending limb of the loop of Henle. The kidneys are the primary regulators of Mg\(^{2+}\) homeostasis [29].

### 1.3.3 Hypomagnesemia

Hypomagnesemia occurs in up to 15% of the population. It is usually mild to moderate and without clinical significance, however, chronic severe hypomagnesemia can eventually lead to hypertension, diabetes mellitus, renal diseases, and cancer [29]. Several causes have been identified such as medication (i.e. cyclosporine, tacrolimus, omeprazole, cetuximab, and cisplatin), and insufficient dietary intake. Inherited disorders which mainly alter the reabsorption on the thick ascending limb of Henle or distal convoluted tubule are also included and have been grouped into two main groups: hypercalciuric, and Gitelman-like hypomagnesemia [29]. Patients can present with neurological symptoms such as cramps, spasms, and seizures or renal symptoms such as nephrocalcinosis [29]. A disturbance in the serum-bone Mg\(^{2+}\) equilibrium can lead to increases in the reabsorption of stored Mg\(^{2+}\) and eventually decreases in bone density. Low Mg\(^{2+}\) levels can affect the normal bone homeostasis by altering the PTH, Vitamin D, and Ca\(^{2+}\) effects on the different cellular functions [35, 36, 37, 38].

### 1.3.4 Hypermagnesemia

In patients with end-stage renal disease, serum Mg\(^{2+}\) level is elevated. This will alter the Ca\(^{2+}\)/Mg\(^{2+}\) balance by altering the formation of the hydroxyapatite crystals which leads to several bone events such as osteomalacia and renal osteodystrophy. Elevated Mg\(^{2+}\) level can affect bone metabolism by altering the PTH function and osteoblastic cell differentiation [37]. It also causes muscle fatigue and weakness and manifests as lethargy, confusion, and coma [29].
1.3.5 The Role of Mg\textsuperscript{2+} in Osteoblastic Differentiation and previous Mg\textsuperscript{2+} works

To study the role of Mg\textsuperscript{2+} in osteoblastic cell differentiation, researchers have been using different techniques [40]. It is widely understood that hypomagnesemia contributes to the osteopenia process by altering the growth and activity of osteoblasts [40]. In rats, low Mg\textsuperscript{2+} levels have been associated with decreases in osteoblastic cell activity [40]. Further, a decrease in the number of osteoblasts secondary to low Mg\textsuperscript{2+} levels has been demonstrated by histomorphometry [41]. The decrease in bone mass as a result of hypomagnesemia seems to correlate with the decrease in bone formation [40]. Thus, the role of Mg\textsuperscript{2+} in bone homeostasis is very crucial.

Mg\textsuperscript{2+} is an indispensable cofactor in more than 300 enzymes that are involved in regulating a variety of cellular functions [40]. It is also an important modulator of the intracellular pH and free calcium concentration, which are crucial for cell motility and proliferation [39]. Therefore, the effects of low and high Mg\textsuperscript{2+} concentrations on the proliferation and differentiation of osteoblastic cells have been studied [39, 40, 59]. Those studies have focused on certain types of osteoblasts (i.e. normal osteoblasts and MG-63 osteoblast-like cells) that were derived either from human or rodent tissues. Yang et al., [48] have evaluated the effect of Mg\textsuperscript{2+} alloys on osteogenic differentiation of human bone marrow-derived mesenchymal cells and found significant increases in the differentiation, viability, and proliferation of the cells as a result of Mg\textsuperscript{2+} ions derived from the alloys.

In order to more fully explore the potential therapeutic role of controlling the extracellular Mg\textsuperscript{2+} in the microenvironment so as to regulate bone formation in normal and/or osteosarcoma cell growth, more information is needed to better understand the role of extracellular magnesium in growth and differentiation of osteoblastic cell types that may represent cells with various inherent differences in their regulatory mechanisms.
Hypothesis:

The metabolic activity, differentiation and mineralization ability of human SaOS-2 osteosarcoma cells including the effects of PDGF are dependent on the concentration of extracellular magnesium.
Chapter 2: MATERIALS AND METHODS

2.1 Cell culture:

All experiments were performed with SaOS-2 human osteosarcoma cells that were purchased from American Type Culture Collection (ATCC). Cells were allowed to grow in a 75cm² culture flask with 6-8 ml of alpha minimum essential medium (alpha-MEM) with 10% fetal bovine serum and 5% antibiotics. Cells were then cultured in 5% CO₂ at 37°C and were harvested weekly with a trypsin solution (0.25%). The medium was changed every 2 to 4 days according to the type of experiment.

2.2 Experimental solutions:

Three experimental solutions were prepared from Mg²⁺-free DMEM/F12 (Sigma-Aldrich, St. Louis, MO) without added Mg²⁺ or with Mg²⁺ added (MgSO₄; Fisher Chemicals) for two final concentrations of 0.8 mM to represent a physiological concentration of Mg²⁺ and 0.01mM to represent a low Mg²⁺ concentration status.

2.3 Platelet-derived growth factor:

PDGF-BB recombinant human protein (10 ug) was purchased from (Thermo Fisher Scientific INC) and stored at -20°C. It was then diluted with McCoy’s media/DMEM media without Mg²⁺ to yield a final concentration of 250 ng/ml PDGF.
2.4 Cell metabolic activity

The MTT 3(4,5-dimethlythiazol-2-yl) 2,5-diphenyltetrazolium bromide assay was used to assess the cell metabolic activity. MTT assay is used to assess the cell growth as a function of metabolic activity. The yellow tetrazolium MTT reagent is reduced by the active metabolic cells under the influence of the NAD(P)H in the cytosolic compartment of the cell. This results in solubilized purple formazan that then can be quantified by a spectrophotometer.

Cells were seeded onto a 15 well plate – 5 for each assigned concentration - at a density of 10,000 cells per well in 100 µl of the medium. After incubation for 48 hours, the cells in the wells were treated with 13 µl of MTT reagent (Sigma-Aldrich). The plate was covered with aluminum foil and incubated in a humidified condition of 5% CO₂, at 37°C for 2-4 hours. After that, 130 µl of the detergent reagent DMSO (dimethyl sulfoxide) was added to each well and incubated for 30-50 minutes. The absorbance was read with Bio-Rad® microplate spectrophotometer (Bio-Rad laboratories, NY, USA) at 540nm wavelength.

2.5 Alkaline phosphatase assay:

The osteogenic differentiating activity was assessed using the Alkaline Phosphatase Assay (ALP). ALP catalyzes the hydrolysis of p-nitrophenol phosphate (pNPP) to p-nitrophenol (pNP). pNPP is colorless but pNP has a strong absorbance at 405 nm which is proportional to the enzyme activity.

Cells were seeded onto a 15 well plate – 5 for each assigned concentration (Mg-free, Mg²⁺ (0.8 mM), and Mg²⁺ (0.01 mM) supplemented media)- at a density of 10,000 cells per well in 100 µl of the medium for 5 different durations: 24, 48, 72, 96, and 120 hours. The cells were then treated with Triton-X-100 for one hour. The cells were incubated with para-nitrophenol phosphate
(pNPP) and 2-amino-2-methyl-1-propanol for an hour. 0.5N NaOH was used to stop the reaction. The absorbance was read at 405 nm on the BioRad Microplate reader.

2.6 Alizarin red assay

To measure the deposition of calcium salts in the cell culture Alizarin red assay using the Alizarin red dye was done. Mineralization is evaluated by assessing the calcium extraction at a low pH point, neutralization with ammonium hydroxide, and colorimetric detection at 570 nm. Calcium ions form a complex with the alizarin red in the chelation process.

The cells were grown in two 12 well plates for 10 and 21 days, respectively, under the influence of both magnesium free and magnesium containing media (500 µl per cell). Upon the end of the incubation time, the cells were washed with PBS and fixed with 70% ethanol for 1hr at 4°C. The cells were stained with 40 mM alizarin red (Sigma Aldrich, #5533) for 10 minutes and washed with sodium acetate buffer (pH 6.3). Cells were washed with PBS until they were free of the stain debris. The cells were then incubated at room temperature for 1 hour with 500 µl of 10% (w/v) cetylpyridinium chloride (CPC- C0732, Fisher Scientific). After that, the dye was removed and 200 µl aliquots of cells were transferred to a 96 well plate to quantify the mineral deposition at 570 nm absorbance.

2.7 Series of experiments

1- The first experiment was performed to evaluate the possible difference in the metabolic activity of SaOS-2 cells when cultured in different magnesium concentration (0.01 mM), (0.8 mM) and no added magnesium for 48 hours.
2- The second set of experiments was conducted to evaluate the difference in the metabolic activity of the cells when both the media with magnesium (0.01 mM), (0.8 mM), and no magnesium were supplemented with PDGF-BB (250 ng/ml). Controls were performed for each magnesium concentration and magnesium free media containing no PDGF. The activity was assessed at 48 hours.

3- The third set of experiments was performed to assess the difference in the cell differentiation potential of the cells in the presence of low magnesium (0.01 mM), high magnesium (0.8 mM), and magnesium free media by means of quantifying alkaline phosphatase activity at time points of 24, 48, 72, 96, and 120 hours.

4- The fourth set of experiments was done to evaluate the deposition of calcium phosphate crystals by the cells under the influence of magnesium free, low magnesium (0.01 mM), and physiological magnesium (0.8 mM) concentrations for 10 and 21 days. Calcium deposition was assessed by the uptake and quantification of the alizarin red dye at the end of the incubation period.

2.8 Statistical analysis:

Obtained data are expressed as mean ± standard deviation (SD). Statistical differences were determined using one-way ANOVA. * = p <0.05.
Chapter 3: RESULTS

3.1 Experiment 1: Influence of extracellular Mg$^{2+}$ on SaOS-2 cells metabolic activity

The first series of experiments were conducted to assess the effects of extracellular Mg$^{2+}$ concentrations on the cellular activity of SaOS-2 cells as assessed with the MTT assay. As shown in Figure 3, after 48 hours of incubation, the cells incubated in medium with no added Mg$^{2+}$ exhibited significantly less activity in comparison to those incubated for the same period of time in 0.01 mM or 0.8 mM Mg$^{2+}$.

**Evaluation of SaOS-2 cells metabolic activity in 48 hours**

![Graph showing cell metabolic activity](image)

**Figure 3**: Evaluation of the cell metabolic activity of SaOS-2 cells using MTT assay. SaOS-2 cells were cultured in 0 (Mo), 0.01, and 0.8 mM Mg for 48 hours. Zero and low Mg$^{2+}$ concentration (0.01 mM) have reduced the cell metabolic activity compared to the physiological Mg$^{2+}$ concentration (0.8 mM). The increase in cell metabolic activity upon exposure to higher Mg$^{2+}$ comparing to zero Mg ($P = 1.22 \times 10^{-10}$) and low Mg$^{2+}$ concentration ($P = 0.0004$) was statistically significant. Data are the mean +/- standard deviation; N=5; ANOVA used for statistical analyses *= Statistically significant compared to M0.
3.2 Experiment 2: *Influence of PDGF and extracellular Mg concentrations on SaOS-2 metabolic activity*

The results in Figure 4 show that when PDGF-BB (250 ng/ml) was added to the incubation media with the two different concentrations of Mg$^{2+}$ or with no added Mg$^{2+}$, the growth factor did not produce any significant effects in SaOS-2 cell activity compared to the appropriate control (non-PDGF added) medium. However, the cells incubated with no added Mg$^{2+}$ or with 0.01 mM Mg$^{2+}$ did exhibit significantly lower activity levels compared to those incubated with 0.8 mM Mg$^{2+}$ in agreement with the data shown in Figure 3.

![Figure 4](image-url)

**Figure 4:** Evaluation of the cell metabolic activity of SaOS-2 cells with different extracellular Mg$^{2+}$ concentrations with and without PDGF. SaOS-2 cells were cultured in zero, 0.01, and 0.8 mM Mg for 48 hours. PDGF-BB was added at 250 ng/ml. At each different Mg$^{2+}$ concentration there was no difference in the cell metabolic activity of SaOS-2 cells comparing PDGF treated to non-PDGF treated cells ($P > 0.05$) although there were significant differences in the activity levels among all three extracellular Mg conditions ($P < 0.05$). Values are the mean +/- standard deviation; $N=5$; ANOVA was used for statistical analyses.
3.3 Experiment 3: *Alkaline phosphatase activity studies*

The differentiation of SaOS-2 cells was evaluated by the measurement of alkaline phosphatase (ALP) activity in the Mg\(^{2+}\) free, low Mg\(^{2+}\) (0.01 mM), and physiological Mg\(^{2+}\) (0.8 mM) concentrations supplemented media for 24 hours, 48 hours, 72 hours, 96 hours, and 120 hours. There was no difference in the alkaline phosphatase activity for the SaOS-2 cell cultures grown for 24, 48, 72 hours among the different Mg\(^{2+}\) concentrations. In contrast, ALP activity was significantly higher for the SaOS-2 cells culture grown for 96, and 120 hours in high Mg\(^{2+}\) concentration in comparison to those grown under low concentrations (0.01 mM) or Mg\(^{2+}\) free media (Figure 5). These results highlight that time is a significant factor in the ALP activity response of SaOS-2 cells to Mg\(^{2+}\).

**Evaluation of SaOS-2 cells differentiation by ALP assay at different periods of incubation**

![Graph showing ALP activity](image)

**Figure 5:** The differentiation of SaOS-2 cells was evaluated by the measurement of alkaline phosphatase (ALP) activity in the Mg\(^{2+}\) free, low Mg\(^{2+}\) (0.01 mM), and physiological Mg\(^{2+}\) (0.8 mM) concentrations supplemented media for 24 hours, 48 hours, 72 hours, 96 hours, and 120 hours. ALP activity was measured at 405 nm and was significantly higher (*) for the SaOS-2 cells grown for 120 hours in high Mg\(^{2+}\) concentration in comparison to those grown under Mg\(^{2+}\) free \((P = 0.0009)\) or low concentrations (0.01 mM) Mg\(^{2+}\) \((P = 0.030)\). Values are the mean +/- standard deviation; \(N=5\); ANOVA was used for statistical analyses.
3.4 Experiment 4: *Evaluation of the change in mineralization using Alizarin red assay*

To determine if extracellular Mg\(^{2+}\) concentration affects SaOS-2 cell mineralization, ARS staining was performed after 10 and 21 days of incubation. SaOS-2 cells that were grown in the zero or low Mg\(^{2+}\) concentration medium displayed decreased mineralization compared to those grown in 0.8 mM Mg\(^{2+}\) (Figure 6). There was a significant increase in mineralization in the high Mg\(^{2+}\) group compared to the low Mg\(^{2+}\) and no Mg\(^{2+}\) groups in the cultures incubated for the 10 day period (*P* = 0.0003). However, there was no significant difference in mineralization between the high Mg\(^{2+}\) group compared to low Mg\(^{2+}\) group in cultures incubated for the 21 day period (*P* = >0.05).

**Evaluation of mineralization at 10 and 21 days using Alizarin red assay**

![Graph comparing mineralization at 10 and 21 days](image)

*Figure 6:* The mineralization capability of SaOS-2 cells was evaluated by the measurement Alizarin red assay in the Mg\(^{2+}\) free, low Mg\(^{2+}\) (0.01 mM), and physiological Mg\(^{2+}\) (0.8 mM) concentrations supplemented media for 10 and 21 days. The absorbance was measured at 570 nm. There was a significant increase in mineralization in the high Mg\(^{2+}\) group compared to the low Mg\(^{2+}\) and M0 groups in the cultures incubated for the 10 days period (*P* = 0.0003). Values are the mean +/- standard deviation; N=5; ANOVA was used for statistical analyses.
Chapter 4: DISCUSSION

Magnesium is the second most abundant intercellular cation and an indispensable cofactor in more than 300 enzymes that are involved in regulating a variety of cellular functions [40]. It is also an important modulator of the intracellular pH and free calcium concentration, which are crucial for cell motility and proliferation [39]. It is widely understood that hypomagnesemia contributes to the osteopenia process by altering the growth and activity of osteoblasts [40]. Therefore, the effects of low and high Mg\(^{2+}\) concentrations on the proliferation and differentiation of osteoblasts have been studied [40, 42, 59]. Those studies were focused on certain types of osteoblasts (i.e. normal osteoblasts and MG-63 osteoblast-like cells) that were derived either from human or rodent tissues. Yang et al., [48] have evaluated the effect of Mg\(^{2+}\) alloys on osteogenic differentiation of human bone marrow-derived mesenchymal cells and found significant increases in the differentiation, viability, and proliferation of the cells as a result of Mg\(^{2+}\) ions derived from the alloys. However, only few studies have investigated the same effect on osteosarcoma cells [40, 42, 48, 60].

In this present study, the osteosarcoma cell line (SaOS-2) was used. The reason we used SaOS-2 cells is that most of the previous studies have used cell lines that are known for their higher differentiation ability such as MG-63 cells or lower differentiation ability such as G-292 cells (Figure 7) [42, 45, 60] whereas SaOS-2 cells show a moderate differentiation level [45]. Our experimental results show consistently that extracellular Mg\(^{2+}\) concentrations play a significant role in the control of metabolic activity and the differentiation of SaOS-2 cells. These results, compared to other osteosarcoma cell lines that were tested with different Mg\(^{2+}\) concentrations, are in a substantial agreement as they show that the physiological Mg\(^{2+}\) concentration (0.8 mM)
promoted the growth of SaOS-2 cells in comparison to the lower Mg\(^{2+}\) concentration (0.01 mM) which significantly reduced the cell growth and differentiation.

Although MTT assay was earlier described as an effective method to evaluate the cell metabolic activity which reflects the growth and proliferation capability of cells it does measure the ability of cells to modify the MTT salt by their mitochondrial dehydrogenase enzymes. Therefore, this assay has limitations in that it is not actually quantifying cellular proliferation and it only reflects metabolic activity that might not be always directly related to cell growth. The results of this study indicated that the level of cellular activity was low in the absence of extracellular magnesium and increased as the Mg\(^{2+}\) concentration was raised to mimic the physiologic level. Similar studies with another human osteosarcoma cell line, MG-63, have found comparable results but with less significant difference between the low and high Mg\(^{2+}\) concentration [39] suggesting that other factors may be involved in different cell lines. The mechanism by which magnesium might have an influence in the regulation of osteosarcoma cells has been explained by several hypotheses. Yang et al., [49] have tested the anti-osteosarcoma effects and mechanisms of” 4-O-amino-phenol-4’-demethylepipodophyllootoxin ether” on the osteosarcoma cell line OS-9901 and have concluded that higher intracellular Mg\(^{2+}\) concentration leads to reduction of the intracellular pH which subsequently induces apoptosis.
Figure 7. Proliferation capacity of 22 osteosarcoma cell lines [45].

Bone growth factors, mainly BMPs, FGF, PDGF, and TGF-B, have an essential role in the differentiation and proliferation of osteoblasts [47]. PDGF is expressed by different cells including osteoblasts and also is one of major component of the platelet rich plasma during bone fracture healing. Moreover, PDGF-BB has been shown to be involved in the stimulation of bone formation and remodeling by increasing proliferation and migration of osteoblasts [39]. It has been also found to enhance DNA and collagen synthesis in rat osteoblasts cultures [39].

Several studies have tested the possible interaction of PDGF and extracellular magnesium concentration in bone cells [39, 50]. The study of Abed et al., [39] suggests that PDGF increases the proliferation of the cells by upregulating the expression of TRPM7 to ensure long term Mg$^{2+}$ homeostasis and promotion of bone cell proliferation and migration. The stimulation of cell proliferation with PDGF was significantly reduced by concentration of Mg$^{2+}$ below 0.01 mM. Hence, the dual role of Mg$^{2+}$ and PDGF in osteoblastic differentiation has been suggested. In a
preliminary study [60] conducted in our lab with another osteosarcoma cell line, G292, low extracellular concentrations of Mg\(^{2+}\) significantly decreased metabolic activity compared to physiological Mg\(^{2+}\) concentrations but the stimulatory effect of PDGF-BB was still present even with no added extracellular magnesium.

To more clearly delineate the relationship between Mg\(^{2+}\) levels and regulation of cell activity and differentiation in osteosarcoma cells, this current study investigated the role of extracellular Mg\(^{2+}\) in the stimulation of SaOS-2 cells with and without treatment with PDGF-BB. Our results suggest that the PDGF effect on cell activity is negligible and no effect was observed with 250ng/ml PDGF after 48 hours incubation in either physiological or low Mg\(^{2+}\) concentration. These findings are in substantial agreement with the those of Celotti et al., [50], who have shown that PDGF has a minimal proliferation effect on SaOS-2 cells compared to other growth factor such as TGF-B. Moreover, our results with PDGF-BB are consistent with the results of another report in which, in comparison to other osteosarcoma cell lines, the expression of PDGF receptors by SaOS-2 cells has been described to be very weak with only a slight expression of PDGF-R alpha and essentially no expression of PDGF-beta [52].
Evaluation of the mineralization of the SaOS-2 cells in different Mg$^{2+}$ concentrations

Producing extracellular bone matrix is the main function of osteoblasts and a characteristic feature of osteosarcoma cells as well. In normal bone remodeling, this process is fundamental to ensure continuous bone mass deposition. While Mg$^{2+}$ deficiency is well known to contribute to osteoporosis by inhibiting the growth of osteoblasts, little is known about its *in vitro* effect on mineralization in osteosarcoma cell lines. Alizarin red assay is a widely used method to assess the mineralization activity of the cells by detecting the calcium phosphate in culture [51]. Studying the effect of Mg$^{2+}$ on osteosarcoma cells mineralization ability, Leidi et al. [40] have found that lower Mg$^{2+}$ concentration (0.01mM) as well as the physiological concentration (0.8 mM) promoted the mineralization while, on the other hand, a higher Mg$^{2+}$ concentration (5 mM) significantly inhibited the deposition of mineral matrix. Interestingly, there was no significant difference between SaOS-2 cells in low and physiologic Mg$^{2+}$ concentration in terms of the amount of mineral matrix deposition. The results of our study are in partial agreement with the study of [40] in that after 21 days of incubation, no significant differences in mineralization were observed with the different extracellular Mg$^{2+}$ concentrations although with the shorter incubation period of 10 days there was a decrease in this parameter when the Mg$^{2+}$ concentration was low. This finding suggests the effect of extracellular Mg$^{2+}$ in SaOS-2 cells mineralization is similar to what has been reported for normal osteoblastic cells and may not be a factor unique to osteosarcoma cell differentiation.

Several reports suggest that Mg$^{2+}$ is intimately involved in the mineralization ability of osteoblasts [43, 59]. Studies in our lab [59] found that the mineralization capability of normal osteoblasts was reduced in the zero concentration Mg$^{2+}$ compared to physiologic concentration suggesting an important role for Mg$^{2+}$ in the mineral matrix deposition of osteoblasts. Moreover,
the role of TRMP7 channels is well characterized in regulating Mg\(^{2+}\) and Ca\(^{2+}\) cellular transportation. Low Mg\(^{2+}\) is reflected in less calcium influx into the cells while high Mg\(^{2+}\) also decreases the influx of Ca\(^{2+}\) as well, however, in a very distinct mode of action [40, 59]. High Mg\(^{2+}\) competes with Ca\(^{2+}\) ions which impairs the calcium-mediated events [40].

**Evaluation of the differentiation of the SaOS-2 cells in different Mg\(^{2+}\) concentrations**

Alkaline phosphatase (ALP) is a crucial component in the bone formation and remodeling processes. It is highly expressed in the outer surface of the plasma membrane. It is also expressed particularly in the early stages of osteoblastic differentiation and through the mineralization phase and decreased when other bone proteins such as osteocalcin increases.

ALP enzymatic activity was used a marker to evaluate the differentiation of SaOS-2 cell in different Mg\(^{2+}\) concentrations. As expected, the results reveal a time-dependent increase in the ALP activity of SaOS-2 cells incubated with the physiological extracellular Mg\(^{2+}\) concentration. In the low concentrations and absence of Mg\(^{2+}\), reduction in ALP activity was noted compared to the physiological levels of Mg\(^{2+}\) although the ALP levels also increased with time of incubation. Leidi et. al. reached the same results with SaOS-2 cells [40] although their study focused on higher, supraphysiological concentrations of Mg\(^{2+}\) that produced significant decreases in this marker of differentiation. It is important to note that similar effects of low Mg\(^{2+}\) concentrations on alkaline phosphatase observed in this present study with SaOS-2 cells were found in studies conducted in our lab with normal human osteoblasts [59], however, the magnitude of the response appeared to be different between the SaOS-2 and normal cells. The SaOS-2 cells were found to be more responsive to the lower concentration of Mg\(^{2+}\) compared to normal osteoblasts and significant decreases in alkaline phosphatase activity were observed in the osteosarcoma cells with the low or
zero Mg\(^{2+}\) concentrations after only longer periods (96 & 120 hours) of incubation. This difference in response between normal and SaOS-2 might be related to the cancerous phenotype of SaOS-2 cells, but more studies of this nature in other osteosarcoma cell lines are necessary before any conclusions of this nature can be made. Our earlier work with the G292 cells did not include studies on the effects of extracellular magnesium on alkaline phosphatase, only metabolic activity, so we have limited observations in this regard. In clinical settings, Mg\(^{2+}\) has enhanced the cell response in the initial stages of osseointegration [46]. This increase in ALP activity is associated with expression of numbers of osteoblastic genes such as BCP and OCN [44, 53]. Moreover, Mg ions have shown a capability to elevate the levels of ligand binding integrins that in turn activate the integrin-mediated intracellular signaling pathways which can positively influence osteoblastic differentiation [44, 46].

In conclusion, our study has shown that Mg\(^{2+}\) is an important factor for the activity of SaOS-2 cells in culture. PDGF seems to have minimal effect on SaOS-2 cells which can be related to the lower expression of PDGFR by the cells [50]. Further, the mineralization capability of SaOS-2 cells under the influence of Mg\(^{2+}\) was not significant and appeared to be highly dependent on time with possibly other cellular contributing factors. Finally, the impact of Mg\(^{2+}\) in the differentiation of SaOS-2 cells as assessed by alkaline phosphatase activity was noted in a time-dependent manner with less distinction between low Mg\(^{2+}\) and physiologic Mg\(^{2+}\) concentration at early stages.

The significance of the findings of this present work is that the results obtained with the SaOS-2 cells add to the understanding of the role of magnesium in sustaining cell activity/proliferation and the potential inhibitory effect of low extracellular magnesium on tumor growth. The results also appear to support the concept that decreased serum magnesium might enhance the response to cancer treatment [54, 55]. In metastatic colorectal cancer, the cetuximab-
associated hypomagnesemia has been correlated with significantly better treatment response [55]. Therefore, hypomagnesemia has been proposed as a simple and inexpensive biomarker of outcome in patients with advanced colorectal cancer [55]. More interestingly, in mice with subcutaneous injections of Lewis lung carcinoma, mammary adenocarcinoma and colon carcinoma cells, a low magnesium-containing diet has shown inhibitory effects of the primary tumor growth that was promptly reversed when magnesium was re-introduced [56]. Moreover, consistent with the studies that show that magnesium has an important role in the DNA repair process, researchers have suggested that decreases in serum magnesium might be considered a potential candidate as a radiosensitizer [55].

Two distinctive mechanisms might contribute to the inhibitory effect of low magnesium on tumor growth: 1) low magnesium induced oxidative stress, and 2) impaired angiogenic switch by suppression of the hypoxia induced factors 1-alpha activity [56].

Modulation of the specific magnesium transporters such as TRPM6 and TRPM7 channels, that have been identified as gatekeepers of systemic and cellular magnesium homoeostasis in normal and tumor cells is promising [54]. Some recent studies have indicated that pharmacological inhibition of TRPM7 channel activity can impair the motility and the growth of some cancer cell types [57, 58]. Moreover, in our lab, preliminary in-vitro assessments of G-292 cells have shown that their cell activity can be downregulated by inhibiting the expression of TRPM 7 with an siRNA directed against the molecule. This suggests that utilizing this approach might be useful for regulation of osteosarcoma activity in vivo as well. Further testing this approach in vitro to regulation of SaOS-2 cell activity should now be included to fully understand the potential of inhibition of TRPM7 for regulation of other osteosarcoma types that are also downregulated by low extracellular magnesium. On the other hand, limitations to this interference approach of
controlling magnesium transporters as well as magnesium concentrations in general in an effort to control osteosarcomas in vivo may exist. A major such limitation is that normal osteoblastic cell activity may also be affected by manipulations of magnesium regulation.

In normal osteoblasts, as reported in a previous work done with primary human calvarial cells, by our group, the effect of low magnesium concentrations on the cell activity was similar to what we found in this present study on SaOS-2 osteosarcoma cells. In addition, TRPM7 channels have been expressed also in normal osteoblasts [39]. Therefore, further studies especially on how to create a low magnesium microenvironment as a therapeutic approach to reduce the tumor growth in patients is necessary with possible local delivery mechanisms that might be designed in an in-vivo preclinical model.
References:


52. McGary EC, Weber K, Mills L, Doucet M, Lewis V, Lev DC, Fidler IJ, Bar-Eli M. Inhibition of platelet-derived growth factor-mediated proliferation of osteosarcoma


