Effect of Mesenchymal Stem Cells Systemic Implantation on Glucocorticoid- Induced Osteoporosis in Male Rats: A Comparative Study with Alendronate

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Abstract: Objectives: The present study aims to assess if mesenchymal stem cell (MSCs) transplantation could be useful in the treatment of glucocorticoid-induced osteoporosis (GIO) in rats and to compare the effectiveness of this therapy with a commonly prescribed bisphosphonate (alendronate). Moreover, it aims to clarify the role of osteoprotegerin (OPG) in the development of GIO**. Methods:** Forty adult male albino rats were divided into four equal groups, ten rats each. The first group (group I) served as healthy control group, while osteoporosis was induced in the rest of the rats by daily intraperitoneal (ip) injection of 20 mg/kg dexamethasone for 3 wk after which the osteoporotic rats were divided into three groups: group II: osteoporotic non treated group, group III: injected once with bone marrow derived-mesenchymal stem cell (BMSCs) into tail vein and group IV was given ip injections of alendronate (ALN) 60 mg/kg in saline every other day for 4 weeks. On the 28th day of BMSCs implantation, blood samples were obtained and sera were separated for estimation of bone turnover markers (osteocalcin and hydroxyproline). Bone samples were collected. Osteoprotegerin gene expression was determined with real time-polymerase chain reaction (RT-PCR). Also, histopathological examination of bone was done. **Results:** In the present study Glucocorticoids-induced osteoporosis is characterized by significant decrease in serum levels of osteocalcin (OC) and OPG gene expression in tibia with significant increase of hydroxyproline (HYP) levels in urine. At the end of the treatment period, both BMSCs and ALN increased serum levels of OC compared to GIO rats. There was no significant difference observed between two treated groups. Both BMSCs and ALN significantly reduced HYP urine levels compared to GIO rats. However, ALN, known as an antiresorptive compound lowered hydroxyproline levels significantly more when compared with BMSCs-treated group. Also, OPG gene expression was significantly higher in the ALN-treated group, compared to GIO group, control group and BMSCs-treated group. Also, BMSCs-treated group showed significant increase in OPG gene expression compared to GIO group but its level still lower than the control level. **Conclusion**: Bone regeneration through induction of BMSCs could promote osteogenesis and provide a rational therapeutic strategy for osteoporosis. However, new approach to increase the homing and retention of the BMSCs to bone is required to augment bone formation and increase bone mass for the treatment of osteoporosis.

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1. Introduction

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone tissue, which leads to enhanced bone fragility and increased fracture risk **(Korkia, 2002).** Osteoporosis is a worldwide health problem and a tremendous economic burden **(Burge *et al.*, 2007).**

There are several secondary causes of osteoporosis, of which the most important is GIO, a consequence of the widespread use of glucocorticoids for a variety of inflammatory conditions **(Devogelaer, 2006; van Staa, 2006 and Woolf, 2007).** However, the precise mechanism of GIO is unclear. Recently, OPG; an osteoclastogenesis inhibitory factor, has been identified as a novel cytokine, which inhibits differentiation and activation of osteoclasts **(Clowes *et al.*, 2005).**

A wide variety of pharmacological interventions have been shown to decrease bone loss in GIO. Proposed treatments to maintain or increase bone density include calcium supplementation, bisphosphonates, hormone replacement therapy, vitamin D, calcitonin, parathyroid hormone, fluoride, testosterone and anabolic steroids **(Adachi *et al.*, 1996; Ringe *et al.*, 1999; Eastell *et al.*, 2000; Boutsen *et al.*, 2001; Crandall, 2002 and Sambrook, 2007).**

Over the past 3 decades, bisphosphonates (BPs) have been developed as potent inhibitors of bone resorption and effective agents for the management of osteoporosis and other bone diseases **(Rodan and Fleisch, 1996).** Decreased osteoclast progenitor development, decreased osteoclast recruitment, and promotion of apoptosis of mature osteoclasts leading to decreased bone remodeling are thought to be the

main mechanisms of the antiresorptive actions of BPs **(Sambrook, 2005**). Alendronate is one of the best and most extensively recommended bisphosphonates in the treatment of osteoporosis **(Prinsloo and Hosking, 2006).**

Several alternative therapeutic approaches have also been considered in recent years, as stem cell therapy **(Ocarino *et al.,* 2010)**. Mesenchymal stem cells (MSCs) are adult stem cells present in a wide variety of tissues that are capable of differentiating into various mesenchymal and non-mesenchymal lineages **(Chamberlain *et al.,* 2007).** In addition, these cells have been shown to play an important role in hematopoiesis **(Dazzi *et al.,* 2006)**, bone physiology **(Bruder *et al.,* 1994)** and in part participate in the pathophysiology of bone diseases **(Oreffo *et al.,* 2005).** Because of the osteogenic differentiation potential and the relative ease of isolation and expansion, MSCs became promising materials for treating various bone degenerative disorders including osteoporosis **(Trounson *et al.,* 2011).**

In light of these observations, the present study aims to assess if mesenchymal stem cell transplantation could be useful in the treatment of glucocorticoid-induced osteoporosis in rats and to compare the effectiveness of this therapy with a commonly prescribed bisphosphonate (alendronate). Moreover, it aims to clarify the role of osteoprotegerin in the development of GIO**.**

2. Materials and Methods:

Animal and Experimental Design

Forty adult male albino rats, weighing 180–200 gm, were used for this study. Rats were kept in plastic cages at room temperature (23-25°C) under a day/night rhythm in our institutional facility with free access to food and water.

Rats were divided into four equal groups, ten rats each. The first group (group I) served as healthy control group, while osteoporosis was induced in the rest of the rats by daily intraperitoneal (ip) injection of 20 mg/kg/day dexamethasone for 3 wk, after which the osteoporotic rats were divided into three groups, group II: osteoporotic non treated group, group III: osteoporotic rats injected once with BMSCs into tail vein and group IV: osteoporotic rats received intraperitoneal (ip) injections of alendronate (ALN) (Merk Sharp & Dohme); 60 mg/kg in saline every other day **(Wezeman *et al.,* 2000)** for 4 weeks. On the 28th day of BMSCs implantation, blood samples were obtained and sera were separated for estimation of bone turnover markers (osteocalcin and hydroxyproline). Bone samples (right tibia, left tibia and right femur) were collected. Osteoprotegerin gene expression was determined with real time-polymerase chain reaction (RT-PCR). Also, histopathological examination of bone was done.

Establishment of Glucocorticoid- induced osteoporosis (GIO) model

After 7 days of acclimatization, thirty rats were injected intraperitoneally with 20 mg/kg/day dexamethasone (Sigma-Aldrich) for 21 consecutive days, to establish osteoporosis. Bone mass loss in these animals was examined by measuring their urine levels of the hydroxyproline. Daily injection of 20 mg/kg dexamethasone for 3 wk was sufficient to induce significant bone loss in these rats **(Lien *et al.,* 2009)**.

BMSCs Preparation, Labeling, implantation and tracing:

1. Preparation of BM-derived MSC

Bone marrow was harvested by flushing the femurs of 6-week-old male white albino rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL). Cells were incubated at 37 °C in 5% humidified CO_2 for 12–14 days as primary culture or upon formation of large colonies. When large colonies developed (80–90% confluence), cultures were washed twice with phosphate buffer saline (PBS) and the cells were trypsinized with 0.25% trypsin in 1mM EDTA (GIBCO/BRL) for 5 min at 37 °C. After centrifugation, cells were resuspended with serum-supplemented medium and incubated in 50 cm^2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures **(Alhadlaq and Mao, 2004)**. MSC in culture were characterized by their adhesiveness and fusiform shape **(Rochefort *et al.,* 2006).**

2. Labeling of MSCs with PKH26

MSCs were harvested during the 4th passage and were labeled with PKH26 dye, which is a red fluorochrome. It has excitation (551nm) and emission (567 nm) characteristics compatible with rhodamine or phycoerythrin detection systems. The linkers are physiologically stable and show little to no toxic side-effects on cell systems. Labeled cells retain both biological and proliferating activity and are ideal for *in vitro* cell labeling, *in vitro* proliferation studies and long *in vivo* cell tracking. In the current work, MSCs were labeled with PKH26 from Sigma Company (Saint Louis, Missouri USA). Cells were centrifuged and washed twice in serum free medium. Cells were pelleted and suspended in dye solution **(Feng *et al.,* 2005 and Munoz-Fernandez *et al.,* 2006)**. After inhalation anesthesia with diethyl ether, 0.5 ml cell suspension was injected intravenously into rat tail

vein. After one month, bone tissue was examined with a fluorescence microscope to detect and trace the cells **(Lein *et al.*, 2009).**

Sample collection and biochemical assays:

The animals were anaesthetized at the end of the experiments and blood samples were obtained from the orbital sinus of overnight fasted rats. Blood was immediately centrifuged at 3000 (rpm) for 20 min. sera were separated and stored at -80 ^{0}C until the day of analysis. In the blood specimens, biochemical bone formation marker; osteocalcin was measured using, enzyme-linked immunoassay kits (Formosa Biomedical kits). Urinary hydroxyproline (HYP) concentration was determined spectrophotometrically using the method of **Neuman & Logan (1950).**

Detection of OPG gene expression by real time PCR

Left tibias were used for analysis. OPG expression was determined by real time-polymerase chain reaction (RT-PCR). Tibias were decalcified prior to paraffinization. For analysis, the technique described by **Pfaffl (2001)** for RT-PCR analysis of DNA and RNA extracted from formalin-fixed and paraffin-embedded biopsies was used.

1-Ribonucleic acid (RNA) extraction and complementary deoxyribonucleic acid (cDNA) synthesis:

Total RNA was isolated from bone tissue homogenates using trizol reagent (TM) (Invitrogen, Carlsbad, California) according to the manufacturer's protocol. The RNA sample was dissolved in RNase-free water and quantified spectrophotometrically. The integrity of the RNA was studied by gel electrophoresis on a 1% agarose gel, containing ethidium bromide. First-strand cDNA synthesis was performed with the SuperScript Choice System (Life Technologies, Breda, the Netherlands) by mixing 2 μg total RNA with 0.5 μg of oligo (dT) 12-18 primer in a total volume of 12 μL. After the mixture was heated at 70° C for 10 min, a solution containing 50 mmol/L Tris HCl (pH 8.3), 75 mmol/L KCl, 3 mmol/L $MgCl_2$, 10 mmol/L DTT, 0.5 mmol/L dNTPs, 0.5 μL RNase inhibitor, and 200 U Superscript Reverse Transcriptase was added, resulting in a total volume of 20.5 μL. This mixture was incubated at 42 ° C for 1 h and then stored at －80° C until further use.

2-Real time quantitative PCR

For real time quantitative PCR, 1 μL of first-strand cDNA diluted 1:10 in RNase-free water was used in a total volume of 25 μL, containing 12.5 μL 2x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 200 ng of each primer. Primers, designed with the Primer Express software package (Applied Biosystems), the sequence for OPG gene (forward 5′- TTG TGT GAC AAA TGT GCT CC-3′, (Reverse 5′- GAC GTC TCA CCT GAG AAG -3′) and GAPDH primers (forward 5′- TTCGACAGTCAGCCGCATCTTCTT-3′, reverse 5′- CAGGCGCCCAATACGACCAAATC -3′). PCR reactions, consisting of incubation in 95° C for 10 min (1 cycle), 94° C for 15 s, and 60° C for 1 min (40 cycles), were performed on an ABI Prism 7900 HT Fast Real Time PCR system (Applied Biosystems). Data were analyzed with the ABI Prism 7900 sequence detection system software (genetic analyzer; version 2.2) and quantified with the comparative threshold cycle method with beta actin as a housekeeping gene reference.

Detection of implanted MSCs

Cells labeled with the PKH26 showed strong red autofluorescence after transplantation in rats, confirming that these cells were actually seeded into the bone tissue (Fig. 1).

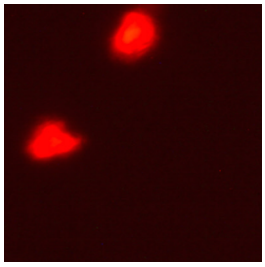

Figure (1): Shows labeling of MSC withPKH26 dye

Histopathological examinations

Autopsy samples were taken from the right tibias of rats in different groups and fixed in 10% formalin saline for twenty four hours and decalcification was occurred on formic acid. Then, the usual procedures of histological examination were done. Sections were stained with hematoxylin and eosin then examined through the electric light microscope **(Banchroft *et al.*, 1996).**

Statistical analysis

Data are expressed as means ± standard deviation (SD). Statistical comparison between different groups were done using one way analysis of variance (ANOVA) followed by Tukey HSD multiple comparison test to judge the difference between various groups. All calculations were performed using the SPSS 16.0 software package. Significance was accepted at $P< 0.05$.

3. Results:

In the present study Glucocorticoids-induced osteoporosis was characterized by significant decrease in serum levels of OC and OPG gene expression in tibia with significant increase of HYP levels in urine, when compared to control group.

At the end of the treatment period, both BMSCs and ALN increased serum levels of OC compared to GIO and control rats, suggesting a stimulation of osteoblast function. However, there was no significant difference observed between the two treated groups.

Both BMSCs and ALN significantly reduced HYP urine levels compared to GIO rats, suggesting decreased bone resorption. However, ALN known as an antiresorptive compound lowered hydroxyproline levels significantly more when compared with BMSCs-treated group. The levels of HYP in the two treated groups were significantly higher than the control group.

OPG gene expression was significantly higher in the ALN compared to GIO group, BMSCs-treated group and control group, suggesting pronounced antiresorptive effect. Also, BMSCs-treated group showed significant increase in OPG gene expression compared to GIO, but its level still lower than the control level.

Table (1): Bone turnover markers in different groups.

GROUPS / Parameters	Control	GIO	BMSCs	ALN
OC (ng/ml)	329.63±1.34 a	155.26±0.75 b	542.91±1.68 c	511.51±2.01 c
HYP (µg/24h)	42.78±1.23 a	159.15±0.65 b	118.33±1.53 c	86.18±1.41 d
OPG (%)	0.16±0.01 a	0.080±0. 02 b	0.13±0.08 c	0.46±0.02 d

Means followed by the same letter within the same row are not significantly different at $p<0.05$ level using Tukey HSD test.

Histopathological Findings:

GIO rats shows, resorption in the cartilaginous structure as well as in the Haversian system with atrophy in the osteoblasts of the shaft of tibia compared to control group (Figs. 2, 3). BMSCs-treated group shows, normalization of the Haversian system with intact osteoblastic clusters. However, bony resorption is still observed in the bony structure of the trabecullae of the tibia (Fig.4). ALN showed greater effects on bone structure compared with BMSCs. ALN had restored architecture of the cartilaginous structure as well as the osteoblasts in the Haversian system (Fig.5).

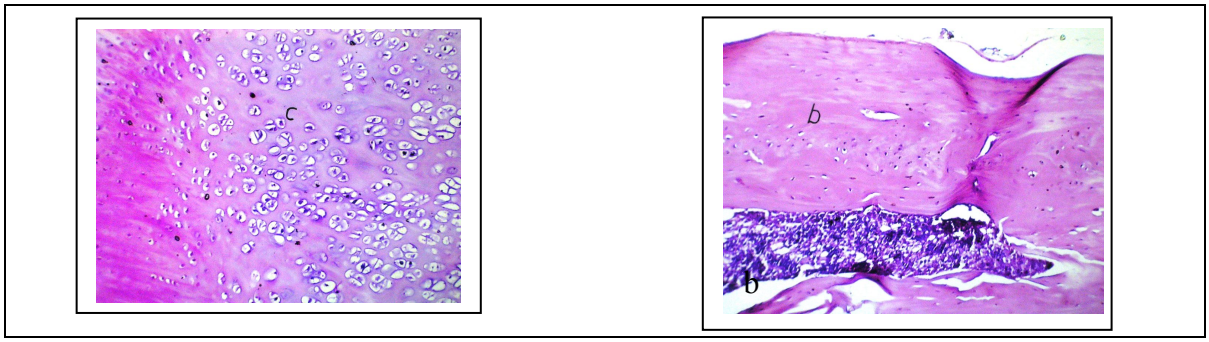

Fig. (2): Histopathological view of bone structure in control group. A: Shows normal histological structure of the cartilaginous surface of the head (C). B: Shows normal histological structure of the Haversian system of the osteoblasts (b), in the shaft with periosteum (P) and bone marrow (bm).

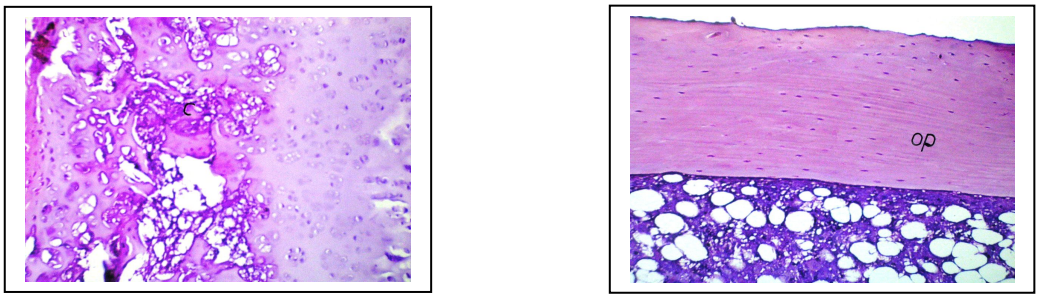

Fig. (3): Histopathological view of bone structure in GIO group. A: Shows resorption of the cartilaginous structure of the head (C). B: Shows osteoporosis and atrophy of osteoblasts (OP).

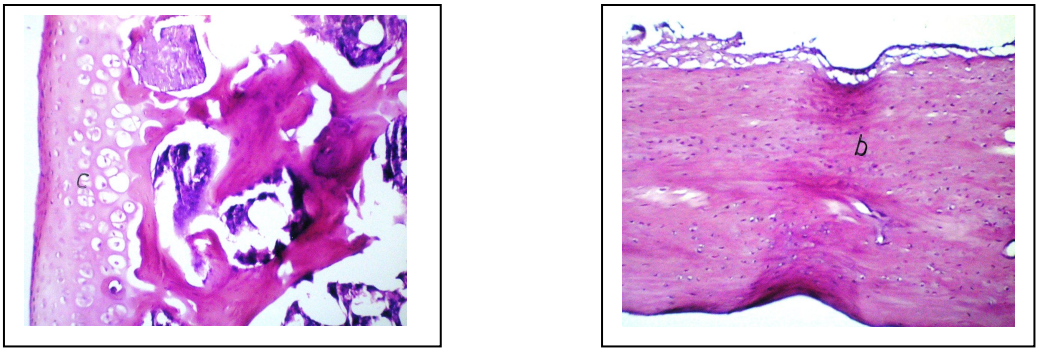

Fig. (4): Histopathological view of bone structure in BMSCs-treated group. A: Shows resorption of the bony structure in trabeculae (C). B: Shows intact histopathological structure of the shaft (f).

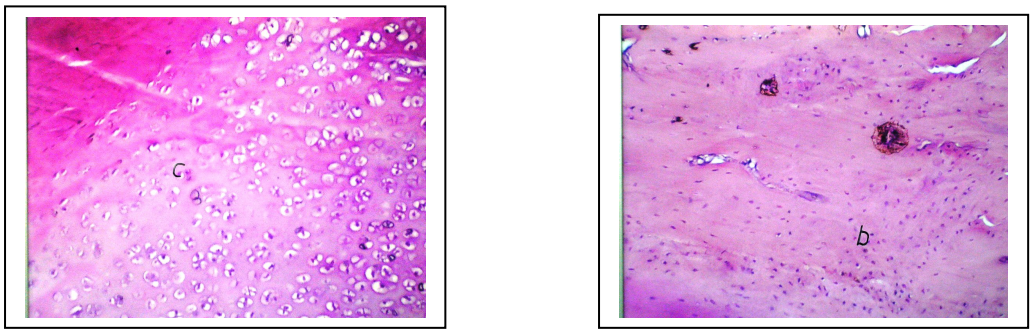

Fig. (5): Histopathological view of bone structure in ALN-treated group. A: Shows normal histological structure of the cartilage in the head (C). B: Shows normal histological arrangement of the osteoblasts in the Haversian system (b).

4. Discussion:

An increasing amount of clinical evidence suggests that the MSCs derived from the adult bone marrow may provide an exciting and promising alternative therapy for repair of bone in skeletal diseases **(Undale *et al.*, 2009).** Osteogenic differentiation of MSCs *in vitro* has been studied, and the applications of MSCs in bone defects in experimental models have been performed with success **(Payushina *et al.*, 2006 and Wang *et al.*, 2006).** Proof of efficacy, however, in the treatment of GIO in comparison with currently available treatments is still lacking **(Teitelbaum, 2010)**. In order to clarify this issue, we investigated the effects of MSCs systemic implantation compared with alendronate in an experimental animal model of GIO.

GIO is particularly serious because it increases the frequency of fractures and seriously impairs patient's activities of daily livings **(Ogoshi *et al.*, 2008).** In the present study Glucocorticoids-induced osteoporosis was characterized by significant decrease in serum levels of OC and OPG gene expression in tibia with significant increase of HYP levels in urine, when compared to control group.

It is generally accepted that glucocorticoids rapidly decrease bone formation and increase bone resorption **(Sasaki *et al.*, 2001 and Canalis *et al.*, 2007).** However, the precise mechanisms have not been defined. Glucocorticoids are thought to directly affect the differentiation, activity and lifespan of osteoblasts and osteocytes **(O'Brien *et al.*, 2004).** Others reported that, Glucocorticoids inhibit expression of genes important for bone formation including those responsible for the production of collagen A1, transforming growth factor-b, fibronectin and insulin-like growth factor-1 **(Iu *et al.*, 2005).**

Recently, it has been suggested that the mechanisms for bone resorption in glucocorticoid therapy probably include activation of important and relevant kinase systems **(Horsch *et al.*, 2007; Soares-Schanoski *et al.*, 2007).** Osteoprotegerin has been identified as a novel cytokine, which acts on bone tissues to increase bone mineral density and volume by decreasing the number of active osteoclasts **(Krakauer, 2008).** The present data provide the novel finding that circulating OPG was significantly suppressed by a short-term administration of glucocorticoids. These results are in agreement with **Weinstein *et al.* (2011)** who reported that, glucocorticoids increased production of receptor activator of nuclear factor-κB ligand (RANKL) and reduced production of OPG, resulting in increased osteoclast recruitment and survival in mice.

Osteoprotegerin is a decoy receptor for, the receptor activator of nuclear factor kappa B ligand (RANKL). By binding RANKL, OPG inhibits nuclear kappa B (NF-κB) which is a central and rapid acting transcription factor for osteoclasts survival and differentiation. OPG can reduce the production of osteoclasts by inhibiting the differentiation of osteoclast precursors (osteoclasts are derived from granulocyte/macrophage-forming colony units) into osteoclasts and also regulates the resorption of osteoclasts in vitro and in vivo. OPG binding to RANKL on osteoblast/stromal cells, blocks the RANKL-RANK ligand interaction between osteoblast/stromal cells and osteoclast precursors. This has the effect of inhibiting the differentiation of the osteoclast precursor into a mature osteoclast **(Blázquez-Medela *et al.*, 2011).**

Histopathological findings of this study Showed that, GIO causes resorption in the cartilaginous structure as well as in the Haversian system with atrophy in the osteoblasts of the shaft of tibia. These findings are in consistent with **Dalle Carbonare *et al.* (2005**) who reported that, glucocorticoid-treated individuals has shown a reduction in bone formation at the cellular and tissue level, resulting in reduced bone volume and a decrease in the number of viable osteocytes. There is also some evidence that glucocorticoids cause thinning of trabecular elements **(Natsui *et al.*, 2006).**

In this study, both BMSCs and ALN succeeded in treating GIO in this experimental rat model: both increased serum levels of OC (bone formation marker) and OPG gene expression in bone compared to GIO rats, suggesting a stimulation of osteoblast function. Both of them also, reduced HYP (bone resorption marker) urine levels, suggesting decreased bone resorption. Moreover, they ameliorated the histological damage caused by glucocorticoids.

Surprisingly, however ALN, a very useful drug for osteoporosis (the gold standard treatment for osteoporosis), had a superior effect on restoring bone structure compared with BMSCs implantation. In addition, ALN known as an antiresorptive compound lowered hydroxyproline levels significantly more and significantly increase OPG gene expression when compared with BMSCs-treated group. Also, it restored architecture of the cartilaginous structure as well as the osteoblasts in the Haversian system. **Wezeman *et al.* (2000)** showed that ALN not only inhibit osteoclastic bone resorption in osteoporosis but also promote bone formation. Others found that bone biopsies from subjects in an alendronate trial showed reduction of markers of bone resorption **(Eslami *et al.*, 2011).** Alendronate also, prevented ovariectomy - induced cancellous bone loss in rats, as evidenced by the reduction of urinary hydroxypyridinoline (DPD) **(Iwamoto *et al.*, 2006).**

Animal studies have indicated the following mode of action. At the cellular level, alendronate shows preferential localization to sites of bone resorption specifically under osteoclasts. The osteoclasts adhere normally to the bone surface but lack the ruffled border that is indicative of active resorption. Alendronate does not interfere with osteoclast recruitment or attachment, but it does inhibit osteoclast activity **(Drake *et al.*, 2008).** Also, bisphosphonates (ALN) may target cells of the osteoblastic lineage in different ways to cause outcomes relative to their stimulatory effect on bone formation. Bisphosphonates stimulate cultured osteoblasts in vitro to release a factor that inhibits osteoclastic activity **(Rizzoli *et al.*, 2010).** It increases collagen synthesis and mineralization, builds cancellous bone mass in ovariectomized rats, stimulates fibroblast growth factor 2 (FGF-2) production in bone marrow cultures, and increases positive calcium balance and bone mass in humans and rats (**Bitto *et al.*, 2009**).

Plotkin *et al.* (1999) reported that alendronate administration abolished the osteocyte and osteoblast apoptosis induced by prednisolone in mice. They suggested that the therapeutic efficacy of ALN in diseases such as glucocorticoid induced osteoporosis may be due, in part, to their ability to prevent osteocyte and osteoblast apoptosis. The antiapoptotic effect of alendronate on osteocytes was associated with a rapid increase in the phosphorylated fraction of extracellular signal regulated kinases (ERKs) which are protein kinase intracellular signalling molecules that are involved in functions including the regulation of meiosis, mitosis, and postmitotic functions in differentiated cells. On the other hand it activates ERKs in osteoblastic cells **(Dobnig *et al.*, 2006).**

In agreement with the present data, **Eslami *et al.* (2011**) showed that administration of alendronate to women was associated with reduced pro-osteoclastogenic RANKL and elevated anti-osteoclastogenic factor, OPG, in bone marrow. The ALN-related changes in serum OPG levels during treatment could result from effects on osteoclastogenesis and osteoclast apoptosis as well as from a direct stimulatory effect on osteoblastic OPG production. These changes in OPG levels may be used to predict the individual response of patients to ALN treatment. Given that OPG is a secreted molecule produced by marrow stromal cells and osteoblasts, it is conceivable that the circulating level of OPG is a meaningful indicator of the effects of bisphosphonates on bone marrow **(Reyes-García *et al.*, 2010).** The histopathological findings of this study showed that ALN had restored architecture of the cartilaginous

structure as well as the osteoblasts in the Haversian system. In another study, histomorphometry in rats showed that alendronate treatment reduces bone turnover (i.e., the number of sites at which bone is remodeled). In addition, bone formation exceeds bone resorption at these remodeling sites, leading to progressive gains in bone mass (**Eslami *et al.*, 2011**).

On the other hand, prolonged use of alendronate (more than 5 years) resulting in suppression of bone turnover (resorption and formation) and the bone may accumulate microdamage which, can result in atypical fractures **(Abrahamsen *et al.*, 2010).**

However, stem cell therapy could be a safe alternative treatment for osteoporosis. Mesenchymal stem cells are easily obtainable from bone marrow by means of minimally invasive approach and can be expanded in culture and permitted to differentiate into the desired lineage **(Rastegar *et al.*, 2010).** Scientists believe that stem cells could halt osteoporosis, promote bone growth and initiate new pathways that control bone remodeling **(Pedram *et al.*, 2010).**

In this study, BMSCs increased serum levels of OC and OPG gene expression in bone. They also, reduced HYP urine levels. Moreover, they ameliorated the histological damage caused by glucocorticoids as they show normalization of the Haversian system with intact osteoblastic clusters. But, resorption is still observed in the bony structure of the trabecullae of the long bone. Unexpectedly, BMSCs had a lesser effect on restoring bone structure compared with ALN. **Lien *et al.* (2009)** also, reported that partial restoration of bone formation in glucocorticoid-induced osteoporotic mice was observed 4 wk after a single intravenous infusion of BMSCs.

Bone marrow stem cells (BMSCs) are pluripotent cells that have been used to facilitate bone repair because of their capability of differentiating into osteoblasts **(Kim *et al.*, 2008).** Others reported that, human MSCs inhibit osteoclastogenesis without cell–cell contact, partly due to constitutive secretion of OPG **(Oshita *et al.*, 2011).** MSCs within bone marrow have a multi-lineage potential and give rise to the following cell types: osteoblasts, chondrocytes and adipocytes **(Halleux *et al.*, 2001).** MSCs undergo osteogenic differentiation in the bone marrow, and mobilization of the osteoblastic progenitors to the bone surface is a crucial step in osteoblast maturation and the formation of mineralized tissue **(Chen *et al.*, 2007).** Once the osteoblastic progenitors are directed to the bone surface, they synthesize a range of proteins, including osteocalcin, osteopontin, bone sialoprotein, osteonectin, type-I collagen and fibronectin that will further enhance the adhesion and maturation of the osteoblasts (**Guan *et al.*, 2012).**

The failure of systemic implantation of MSCs to achieve the expected results in osteoporosis models suggests that, the local 'catabolic' environment such as old age and osteoporosis does not provide the required stimulus to the MSCs for their differentiation down the osteoblast lineage **(Trounson, 2009).** It was generally assumed that factors released upon tissue damage or apoptosis mobilize and recruit stem and progenitor cells to the damaged site where they proliferate and differentiate, eventually replacing the damaged tissues. However, it is well known that the number of BMSCs with osteogenic potential decreases in patients with old age, osteoporosis and metabolic diseases. It may be necessary to either combine MSCs with an osteoinductive carrier material to direct the cells to the bone surface for osteogenic differentiation or provide an autologous stimulus by expressing a cell differentiation-inducing agent into the cells (MSC-based gene therapy) in order to achieve therapeutically significant bone regenerative effects **(Pedram *et al.*, 2010). Guan *et al.* (2012),** found that Directing mesenchymal stem cells to bone by attaching them to ALN through specific ligand, will augment bone formation and increase bone mass. In addition, the use of intra-bone marrow injection of differentiated BMMSCs as a simple surgical technique offers a new perspective for local treatment of osteoporosis **(Ocarino *et al.*, 2010).**
Conclusion, Bone regeneration through induction of BMSCs could promote osteogenesis and provide a rational therapeutic strategy for osteoporosis. However, systemic infusions of BMSCs *in vivo* do not achieve the desired osteogenic response in bone because of the limited ability of MSCs to home to the bone surface unless they have infused after certain conditions, such as injuries. New approach to increase the homing and retention of the MSCs to bone is required to augment bone formation and increase bone mass for the treatment of osteoporosis.

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