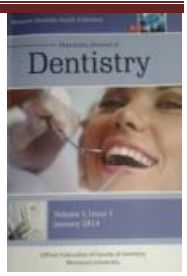
Effects of Bone Marrow Derived Mesenchymal Stem Cells Versus Calcitonin on Mandibular Bone Defects in Osteoporotic Albino Rats



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Abstract:

Background: Osteoporosis is a worldwide health problem with serious impact on the human population, particularly the post-menopausal women and elderly males as well. It causes reduced bone strength, delayed healing and increased liability to fractures. Different therapeutic options are routinely used for prevention and treatment of osteoporosis, calcitonin is considered one of the most currently used antiresorptive drugs. Recently, stem cell-based therapies have gained considerable clinical attention due to their potential and promise to treat a wide range of diseases including osteoporosis.

Objectives: to evaluate and compare the biological effects of bone marrow-derived mesenchymal stem cells versus calcitonin on healing of surgically induced mandibular bone defects performed on osteoporotic rats.

Materials and Methods: Fifty-seven healthy female albino rats weighing approximately 180-200 g were used in the study. Three of them were intramuscularly injected with saline once a week for five weeks then a unilateral surgical mandibular bone defect was created in the diastema region and they were considered negative control, while the other rats were intramuscularly injected with 7 mg/kg body weight dexamethasone sodium phosphate to induce osteoporosis-like condition then they were divided into three groups each included 18 rats. A unilateral surgical mandibular bone defects were created in rats of each group, one group did not receive any treatment and it was considered positive control group, the bone defects of another group were filled with absorbable hemostatic gelatin sponge seeded by 0.5×10^6 bone marrow mesenchymal stem cells (BMSCs) while the bone defects of the last group were filled with absorbable hemostatic gelatin sponge loaded by 10 international unit of injectable synthetic salmon calcitonin. One rat was euthanized at the end of the 1st, 2nd and 4th weeks postsurgically from group I while six rats from the other experimental groups were euthanized at the same timepoints. Then their mandibles were surgically removed and processed for histological analysis.

Results:

Histological results:

Rats of negative control group showed normal healing stages starting by formation of small amount of osteoid tissue at the defect peripheries surrounded by a mass of granulation tissue at the end of the 1st week postsurgically. Two weeks after bone defect creation, the amount and thickness of the formed woven bone increased as detected by Haematoxylin and Eosin stain also the degree of bone maturation and calcification increased as revealed by Masson's Trichrome. By the end of 4 weeks, a network of thicker well- organized bone trabeculae with narrower bone marrow cavities and increased maturity was formed.

The bone healing in the rats of the control positive group was obviously delayed when compared to negative control group, also, the formed bone was of poorer quality, quantity and maturation at the same timepoints.

The quantity, quality and maturation of the bone formed in the group treated by BMSCs seemed to surpass that of the other three groups at the same timepoints.

The histological results of the group treated by salmon calcitonin were better than that of the control groups at all timepoints but were comparable to that of stem cells' group particularly at 1 week timepoint, but the maturation of bone was obviously better in salmon calcitonin group than the control groups at 2 and 4 weeks timepoints.

Conclusions:

Based on the previous results it was concluded that both BMSCs and salmon calcitonin are capable of treating mandibular bone defects in osteoporotic conditions but the bone regeneration in case of using BMSCs surpasses that when using calcitonin at same conditions. This was revealed in terms of better bone quantity, quality and maturation in favor of BMSCs

Introduction

Osteoporosis is a global problem that affects adversely to the post-menopausal women and elderly men as well. The condition affects both sexes and all races, but to different degrees [(1)].

Osteoporosis is defined as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk with minimum trauma [(2)].

The pathogenesis of osteoporosis stems from an improper balance between the processes of bone formation, carried out by the osteoid-secreting osteoblast, and bone resorption, achieved by the proteolytic bone-digestive osteoclast [(3)].

Animal studies have shown that osteoporosis can cause a significant reduction in fracture callus size, bone mineral density (BMD), and mechanical strength [(4)]. Histomorphologically, new bone trabeculae in osteoporotic models are arranged in a loose and irregular fashion, demonstrating the poor quality of the new bone formed [(5)].

Mandibular osteoporosis represents a problem to some dental specialties such as periodontics, maxillofacial surgery, prosthesis, orthodontics and implantodontics, because the success of these specialties largely depends on the maintenance of bone tissue quality and quantity [6].

Different therapeutic options are routinely used for prevention/treatment of osteoporosis. However, their side effects and benefits are under question. The common therapeutic options include anabolic agents and antiresorptive agents. Bisphosphonates, hormone replacement therapy (HRT), raloxifene, and calcitonin are antiresorptive agents, while parathyroid hormone (PTH) is an anabolic agent [7].

Calcitonin is recognised to be among the effective medications for its inhibitory effect on bone resorption [8]. Also, several clinical trials approved the anabolic effects of calcitonin on bone with small but significant increase in bone mineral density after calcitonin administration over 3-5 years [9, 10].

The systemic use of calcitonin has been hindered by poor patient compliance to parenteral and intranasal treatments, whereas orally administered calcitonin has faced safety and efficacy problems [11]. However, the positive effects of calcitonin could still be utilized in osteoporotic bone by local delivery.

During the last decade stem cell-based therapies have gained considerable clinical attention due to their potential and promise to treat a wide range of diseases. Since embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are associated with a number of safety and ethical issues, most of the clinical trials involving stem cell-based therapies use MSCs as the cell source. This is particularly true for clinical applications of stem cells in the musculoskeletal system [12].

Mesenchymal stem cells (MSCs) are thought to be essential for bone healing because of its ability to differentiate in vitro into both chondrocytes and osteocytes, and provide great potential for tissue engineering [13].

MSCs could be collected from various tissues, for example bone marrow, adipose, amniotic fluid, umbilical cord blood and even breast milk [14-17]. Among these origins, bone marrow-derived MSCs are the most commonly utilized stem cells for bone repairing, both in research and clinical practice [18].

In this context, calcitonin as well as bone marrow-derived MSCs may be effective in the treatment of bone defects that occur in osteoporotic patients.

Thus, this study was conducted to demonstrate and compare the biological effects of bone marrow-derived MSCs versus calcitonin on healing of surgically induced mandibular bone defects performed on osteoporotic rats.

Materials and Methods:

Experimental model

Fifty-seven albino rats with an average weight of 180-200 gm were used in this study. All rats were kept under the same nutritional and environmental conditions. All experimental procedures were performed under protocol of ethical committee of Faculty of Dentistry, Mansoura University, Egypt.

Group I (n=3) (negative control group)

Surgical mandibular bone defect was created after intramuscular injection of saline once a week for 5 weeks and left for bone healing without any treatment.

Group II (n=18) (positive control group)

Surgical mandibular bone defect was created after osteoporosis induction according to Pinto Ads et al. [19] and left for bone healing without any treatment.

Group III (n=18) (osteoporotic group treated with BMSCs)

Surgical mandibular bone defect was created after osteoporosis induction then the defect was filled with gelatin sponge seeded by 0.5×10^6 bone marrow derived mesenchymal stem cells [20].

Group IV (n=18) (osteoporotic group treated with salmon calcitonin)

Surgical mandibular bone defect was created after osteoporosis induction and then the defect was filled with 10 IU (international unit) of injectable synthetic salmon calcitonin (Miacalcic,100 IU) carried by gelatin sponge [21].

Osteoporosis induction

The induction of osteoporosis was done by intramuscular administration of dexamethasone sodium phosphate at a dose of 7 mg/kg of body weight, once a week, for 5 weeks, [19] in all groups, except in the negative control group.

Isolation and culture of BMSCs were performed according to protocol of Lotfy et al. in 2014 [22].

Surgical Procedures

All animals included in this study were anaesthetized using intraperitoneal injection xylazine (25mg/kg) body weight and ketamine (75mg/kg) body weight. The skin of the submandibular region was shaved and disinfected with alcohol 70%.

A linear incision was created through the skin paralleling the inferior border of the left mandible then surgical bone defect was created at the mandible between the incisors and the molar region (diastema) buccally with a rose head bur in a slow-speed dental drill along with continuous cooling with saline to avoid overheating.

The bone defects created in animals, which were supposed to receive treatment, were filled with the therapeutic agents before suturing.

Evaluation

Conventional histological analysis

One rat was euthanized at the end of the 1st, 2nd and 4th weeks postsurgically from group I while six rats from the other experimental groups were euthanized at the same timepoints. Then their mandibles were surgically removed and processed for histological staining with Haematoxylin and Eosin stain and Massons' Trichrome stain.

Histochemical analysis

For detection of collagen fibers Masson's Trichrome stain was used. According to its results histomorphometric analysis was conducted.

Statistical analysis

Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 23.0 to obtain

Descriptive data:

Descriptive statistics were calculated in the form of Mean ±Standard deviation (SD).

Analytical statistics:

In the statistical comparison between the different groups, the significance of difference was tested using One way ANOVA (analysis of variance) which was used to compare between more than two groups of numerical (parametric) data followed by post-hoc tukey. A *P* value <0.05 was considered statistically significant (S).

assay.

Results:

Histological analysis:

Haematoxylin and Eosin results:

Group I (negative control)

Rats of negative control group showed normal healing stages starting by formation of small amount of osteoid tissue at the defect peripheries surrounded by a mass of granulation tissue at the end of the 1st week postsurgically. Two weeks after bone defect creation, the amount and thickness of the formed woven bone increased. By the end of 4 weeks, a network of thicker well- organized bone trabeculae with narrower bone marrow cavities and increased maturity was formed **(Fig. 1 A, B & C)**

Group II (positive control)

One week postsurgically small amount of osteoid tissue was formed at the defect margin and was surrounded by large mass of granulation tissue, then, after 2 weeks the osteoid tissue started to increase but still of lesser quantity than that of the negative control group. By the end of the 4th week a network of bone trabeculae was formed however, the formed bone was of poor quality as the trabeculae were of thin thickness and were not connected regularly and enclosed wide bone marrow cavities **(Fig. 1 D, E & F)**.

Group III (osteoporotic group treated by stem cells)

After the first week of healing, the amount of formed osteoid tissue was greater than that of the other three groups and the tissue was radiating perpendicular to the old bone, the tissue was interlaced forming a network surrounded by granulation tissue. After two weeks the trabecular network was almost filling the bone cavity and enclosing relatively narrow marrow cavities. After four weeks, the trabeculae became thicker and more organized when compared to the other three groups **(Fig. 1 G, H & I)**.

Group IV (osteoporotic group treated by salmon calcitonin)

The formed woven bone was of greater amount than that of the control groups but of lesser amount than that of stem cells' group at all timepoints **(Fig. 1 J, K & L)**.

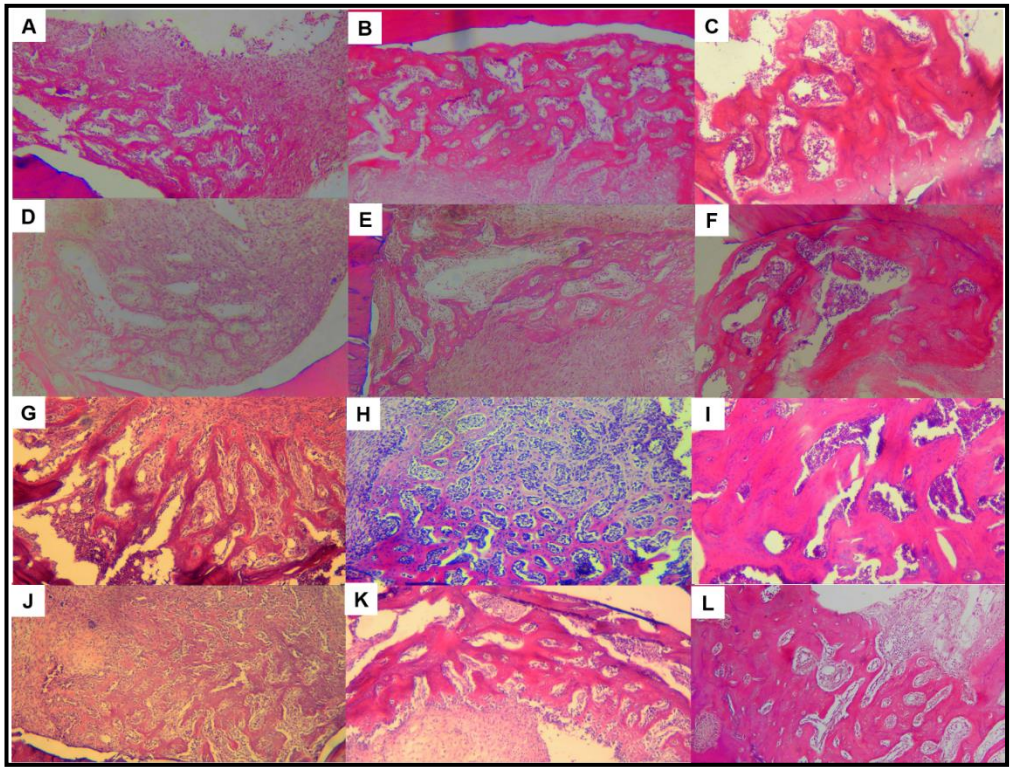

Figure 1: Photomicrograph showing the bony cavities in (A) group I after 1 week, (B) group I after 2 weeks, (C) group I after 4 weeks, (D) group II after 1 week, (E) group II after 2 weeks, (F) group II after 4 weeks, (G) group III after 1 week, (H) group III after 2 weeks, (I) group III after 4 weeks, (J) group IV after 1 week, (K) group IV after 2 weeks and (L) group IV after 4 weeks.

Histomorphometric results

Histomorphometric analysis of the Massons' Trichrome stain slides was done to detect collagen fibers in newly formed bone percentage to the total area. The collected data was statistically analyzed. The resulted data was tabulated (table.1). This table represent the comparison between the four groups at different time of euthanization.

Table (1): Comparison of Percent area among different studied groups

		GROUPS				
		Control -ve	Control +ve	Calcitonin treated	Stem cell treated	P
Week1	Mean	12.02	11.48	16.84	17.20	<0.001*
	±SD	2.405	2.296	3.368	3.439	
	Posthoc		P1=0.97	P1=0.004* P2=0.001*	P1=0.002* P2=<0.001* P3=0.99	
Week2	Mean	9.059	12.79	20.89	24.74	<0.001*
	±SD	1.812	2.557	4.177	4.948	
	Posthoc		P1=0.11	P1=<0.001* P2=<0.001*	P1=<0.001* P2=<0.001* P3=0.095	
Week4	Mean	9.690	12.22	7.556	6.257	<0.001*
	±SD	1.938	2.443	1.511	1.251	
	Posthoc		P1=0.02*	P1=0.06 P2=<0.001*	P1=0.001* P2=<0.001* P3=0.4	

*SD: standard deviation P: Probability *: significance <0.05*
Test used: One way ANOVA followed by post-hoc tukey
P1: significance relative to Control -ve group
P2: significance relative to Control +ve group
P3: significance relative to Calcitonin treated group

Discussion:

Osteoporosis is a worldwide problem that affects wide range of people particularly elderly men and postmenopausal women, it leads to reduced bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk with minimum trauma [1, 2].

Recently, stem cell-based therapies have gained considerable clinical attention since their use in treating various diseases has shown promising results. Mesenchymal stem cells (MSCs) in particular are thought to be crucial for bone regeneration because of its ability to differentiate in vitro into both osteocytes and chondrocytes, and provide great potential for tissue engineering [13].

Various drugs are available for inhibiting bone resorption among which is calcitonin which is vastly used to treat osteoporosis as it inhibits bone resorption and is capable of relieving pain [8].

To our knowledge, this is the first study to examine and compare the biological effects of BMSCs versus calcitonin on healing of surgically induced mandibular bone defects performed on osteoporotic rats.

The specimens were prepared and stained by Haematoxylin and Eosin stain for routine examination and Masson's Trichrome stain for collagen detection. In the negative control group, after the 1st week the cavity was filled with granulation tissue and very small amount of osteoid tissue at the defect margins, after the 2nd week more osteoid tissue started to radiate from the cavity margin and was surrounded by a mass of granulation tissue. After 4 weeks the bone trabeculae became more thick and organized enclosing large marrow spaces. These results were in accordance with Martin et al. (2010) and Richard J et al. (2010) [23, 24].

In the control positive group, One week postsurgically scanty amount of osteoid tissue was detected at the cavity margins and was surrounded by large mass of granulation tissue, then, after 2 weeks the osteoid tissue started to increase but still of lesser quantity than that of the negative control group. By the end of the 4th week a network of bone trabeculae was formed however, the trabecular number decreased and the formed bone was of poor quality as the trabeculae were of thin thickness and were not connected regularly furthermore, they enclosed wide cavities filled with fatty bone marrow. These results were consistent with results mentioned by Yi-Xin et al. (2011) and Richard J et al et al. (2010) [24, 25].

In the BMSCs treated group, After the first week of healing, the amount of formed osteoid tissue was greater than that of the other three groups, the tissue was interlaced forming a network surrounded by granulation tissue. After two weeks the trabecular network was almost filling the bone cavity and enclosing relatively narrow marrow cavities. After four weeks, the trabeculae became thicker and more organized when compared to the other three groups. These results were in accordance with Lei et al. (2012) who used autologous BMSCs to enhance osteoporotic bone defect healing in long-term estrogen deficient goats and Tang et al. (2006) who used

BMSC modified by human bone morphogenetic protein-2 gene transfer to repair mandibular bone defect in osteoporotic rats [26, 27].

In the calcitonin treated group, the formed woven bone was of greater amount and quality than that of the control groups at all timepoints. But when compared to stem cells' group the formed bone was slightly lesser in amount and maturation. The effects of locally delivered calcitonin on osteoporotic bone healing are not thoroughly investigated however, few studies have confirmed their promising results in treating such cases. Li et al. (2007) developed a novel design of salmon calcitonin loaded injectable calcium phosphate cement, this design can be promising in terms of providing an effective treatment for osteoporosis induced bone defects [28].

Conclusion:

Based on the previous results it was concluded that both BMSCs and salmon calcitonin are capable of treating mandibular bone defects in osteoporotic conditions but the bone regeneration in case of using BMSCs surpasses that when using calcitonin at same conditions. This was revealed in terms of better bone quantity, quality and maturation in favor of BMSCs.

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