



Journal of Medical Sciences

ISSN 1682-4474

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>



Research Article

Role of Mesenchymal Stem Cells in Bone Healing of Rat Bisphosphonate-induced Osteonecrosis of the Jaw

¹Samar Ali Altowity, ²Amal Hassan Abdel-Rahman, ²Rehab Fawzi Kasem and ³Mohammed Mahmoud Elsebaie

¹Department of Oral and Maxillofacial Pathology, Faculty of Dental Medicine, IBB University, IBB, Yemen

²Department of Oral and Maxillofacial Pathology, Faculty of Dental Medicine, Cairo University, Giza, Egypt

³Department of Biochemistry, Faculty of Medicine, Cairo University, Giza, Egypt

Abstract

Background and Objective: Bisphosphonates (BPs) have become the primary class of drugs prescribed for the management of bone pathologies associated with excessive bone resorption. Their use has been associated with significant side effect, Bisphosphonate-Related Osteonecrosis of the Jaws (BRONJ). The conventional non-surgical and surgical approaches in several BRONJ lesions are sometimes insufficient, therefore, alternative therapies that are safe and that enhance the osteogenesis and angiogenesis are needed. Mesenchymal stem cells (MSCs) have great potential for clinical therapy and have many applications in various fields of regenerative medicine. This study was conducted to evaluate the effect of MSCs on the healing (regenerative) potentials of the bone in bisphosphonate-induced osteonecrotic teeth socket. **Materials and Methods:** To generate BRONJ-like model, twenty-four adult male rats were received zoledronic acid (Zometa, 0.1 g kg⁻¹ b.wt., twice per week) and dexamethasone (5 mg kg⁻¹ b. wt., once in week) intraperitoneally. At 4 weeks after drugs injection, unilateral left mandibular first molar was extracted and at 3 weeks after teeth extraction, rats were divided randomly into two groups; MSCs group (treated by MSCs topical application) and control group (left without treatment). After euthanization, the mandible of all rats were dissected out and prepared for histopathological examination using H and E staining, Masson trichome as well as immunostaining with osteopontin. **Results:** Signs of bone healing and regeneration were significantly detected in osteonecrotic teeth sockets which were treated with topical application of MSCs in comparison to control group. **Conclusion:** MSCs topical treatment approach might help in restoring, maintaining or improving defective tissue functions that have been compromised by BRONJ.

Key words: Bisphosphonates, bisphosphonate-related osteonecrosis of jaw, mesenchymal stem cell, osteopontin, teeth socket

Citation: Samar Ali Altowity, Amal Hassan Abdel-Rahman, Rehab Fawzi Kasem and Mohammed Mahmoud Elsebaie, 2018. Role of mesenchymal stem cells in bone healing of rat bisphosphonate-induced osteonecrosis of the jaw. J. Med. Sci., 18: 87-95.

Corresponding Author: Rehab Fawzi Kasem, Department of Oral and Maxillofacial Pathology, Faculty of Dental Medicine, Cairo University, Giza, Egypt

Copyright: © 2018 Samar Ali Altowity *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bisphosphonates (BPs) are anti-bone resorptive drug used routinely to decrease osteoclast-mediated bone loss in osteoporosis, multiple myeloma, paget's disease and complications of metastatic disease¹.

Bisphosphonate-Related Osteonecrosis of the Jaw (BRONJ) is a well-known, severe side effect of BPs treatment and occurs in a patient who had current or previous treatment with BPs, has exposed or necrotic bone in the maxillofacial region that persisted more than 8 weeks and has no history of radiation therapy in the jaws. Risk factors for BRONJ include invasive dental procedures, infections and mechanical trauma to the jawbone. These factors are dependent on the dose and duration and the medical condition for which the BPs is prescribed².

Most attempts to control this complication have not been successful, conservative non-surgical approaches have been recommended in managing early stages of BRONJ patients that only slow disease progression, but do not cure the disease³. Standard surgical approaches are indicated in advanced BRONJ, however, further enlarging of the bone defects have been reported⁴. So, the establishment of appropriate and effective approach to prevent and treat BRONJ has been awaited and considered as an urgent issue for patients using BPs.

The concept that mesenchymal stem cells (MSCs) can differentiate and contribute to the regeneration of a variety of non-hematopoietic lineages in multiple parts of the body, has provoked great interest for its potential clinical applications⁵. The MSCs have an osteogenic and chondrogenic potential as well as MSCs are able to contribute to vasculogenesis and angiogenesis with anti-inflammatory and immunomodulatory properties providing vascular and tissue repair^{6,7}.

Several studies were exploited MSCs therapeutic effects in the treatment and prevention of BRONJ. The MSCs systemic injections and transplantation have been studied in animals⁸⁻¹³ and humans^{14,15} with encouraging results, thus this study was performed to investigate the effect of topical application of MSCs in enhancement of bone healing in a rat bisphosphonate-induced osteonecrotic teeth sockets.

MATERIALS AND METHODS

Experimental animals: Three to four months old male Sprague-Dawley (SD) albino rats (n = 24) with a mean

weight of 220 g (range, 190-250 g) were purchased from the animal house, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

Bisphosphonate (zoledronic acid, Zometa® Injection):

The following data were provided in the prescribing information sheet supplied with product: 4 mg/5 mL Zometa (Vial, Novartis Pharma and Basel, Switzerland) was concentrated for infusion allowing withdrawal of 5 mL of concentrate (equivalent to 4 mg zoledronic acid, ZA). During use, this concentrate was diluted in 100 mL of sterile 0.9% sodium chloride or 5% dextrose injection.

Sample size, housing and allocating of experimental animals:

Sample size calculation was done using the comparison of improvement of healing potential of BRONJ between cases treated with topical application of MSCs and control groups by comparing two proportions from independent samples using corrected chi squared test, the α -error level was fixed at 0.05 and the power was set at 80%. Accordingly, the optimum sample size was 12 cases in each arm (using power and sample size (PS) calculations software, version 3.0.11 for MS Windows (William D. Dupont and Walton D. Vanderbilt, USA).

Animals were housed in stainless-steel cages as three rats per cage and individually numbered using tails punches. The cages were placed in a room with filtered air at a temperature $25 \pm 2^\circ\text{C}$. A 12 h light/dark cycle was maintained. The rats were fed a normal rodent diet and water. The rats were randomly distributed by sequence generation computerized program (<https://www.random.org>).

Generation of rat BRONJ-like models

Bisphosphonate administration: All the experimental animals were acclimatized for 4 days before beginning of the induction. During 4 weeks prior to teeth extraction, all SD rats weighed every Monday and Thursday and injected intraperitoneally with zoledronic acid ($0.1 \text{ g kg}^{-1} \text{ b.wt.}$, twice per week to generate BRONJ like animal model and dexamethasone (Dex, $5 \text{ mg kg}^{-1} \text{ b.wt.}$, once in week) as co-medication for lowering the immunity thus acceleration of BRONJ induction¹⁶⁻¹⁸.

Surgical intervention: After 4 weeks of the induction therapy by Zometa, the surgical extraction procedures were done according to the previous studies¹⁸⁻²¹.

Mesenchymal stem cells preparation: Two weeks after teeth extraction, MSCs was prepared *in vitro* as follows²²:

- **Isolation of bone marrow-derived MSCs from rats:** Bone marrow was harvested by flushing the tibiae and femurs of 6 weeks old male white albino rats by a 22-gauge syringe filled with Dulbecco's Modified Eagle's Medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine medium (GIBCO/BRL)
- Nucleated cells 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 CO₂ for 12-14 days as primary culture or upon formation of large colonies
- When large colonies developed (80-90% confluence), cultures washed twice with phosphate buffer saline (PBS) and cells were trypsinized with 0.25% trypsin in 1 mM EDTA (GIBCO/BRL) for 5 min at 37°C. After centrifugation (at 2400 rpm for 20 min), cells resuspended with serum supplemented medium and incubated in 50 cm² culture flask Falcon. The resulting cultures referred to as first-passage cultures
- **Morphological identification of BM-derived MSCs:** The MSCs in culture were characterized by their adhesiveness and fusiform shape and by detection of CD29 (one of the surface markers of rat MSCs (Fig. 1, 2)
- **RT-PCR detection of *CD29* gene expression:** Total RNA was extracted from cells using RNA easy Purification Reagent (Qiagen, Valencia, CA) and then a sample (1 µg) was reverse transcribed with M-MLV (Moloney-Murine Leukemia virus) reverse transcriptase (RT) for 30 min at 42°C in the presence of oligo-dT primer
- Polymerase Chain Reaction (PCR) performed using specific primers (UniGene Rn.25733) forward: 5'-AA TGTTTCAGTGCA GA GC-3' and reverse: 5'- TTGGGAT GA TGTCGGGAC-3'. PCR was performed for 35 cycles, with each cycle consisting of denaturation at 95°C for 30 sec, annealing at 55-63°C for 30 sec and elongation at 72°C for 1 min, with an additional 10 min incubation at 72°C after completion of the last cycle
- To exclude the possibility of contaminating genomic DNA, PCRs run without RT. The PCR product was separated by electrophoresis through a 1% agarose gel, stained and photographed under ultraviolet light

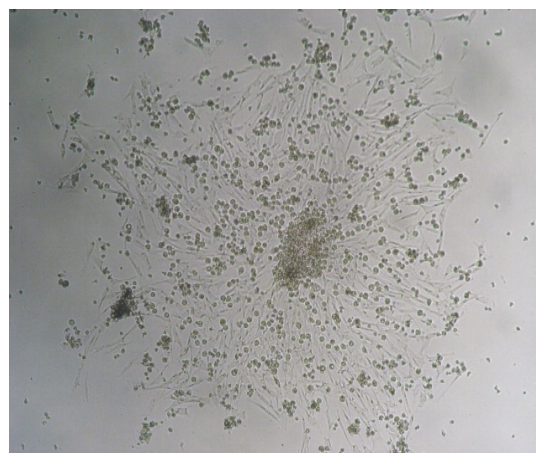


Fig. 1: Mesenchymal stem cells in culture, characterized by their spindle shape

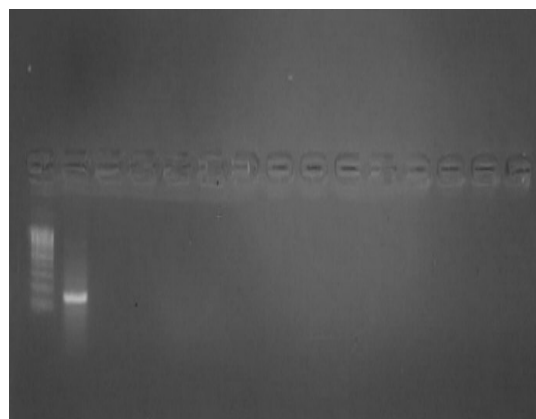


Fig. 2: An agarose gel electrophoresis shows PCR products of *CD29* gene expression in MSC culture (261 bp) (as a molecular marker for rat MSCs)

Lane M: DNA marker with 100 bp, Lane 1: MSCs culture

Clinical observation before MSCs application: After 3 weeks of teeth extraction, the hallmarks of osteonecrosis of the jaw were detected visually as unhealed extraction socket, no soft tissue coverage (open cavity) or white to opaque exposed bone at the site of extraction sockets^{8,12}.

MSCs topical application: After surgical debridement of teeth sockets (removing of food residues with chlorhexidine irrigation), all animals were randomly [by sequence generation computerized program (<https://www.random.org>)] divided into two groups:

- **Control group:** Potential osteonecrotic sockets were left untreated (n = 12)

- **MSCs group:** Potential osteonecrotic sockets treated with topical application of bone marrow derived MSCs (n = 12). The MSCs preparation is applied topically as a gel into the teeth sockets by syringe with metal needle of 16 gauges, then the sockets were closed by interrupted suturing (4-0 resorbable sutures)
- Six animals from each experimental group selected randomly euthanized at 14 and 28 days intervals following MSCs topical application by an overdose of anesthetic solution (1 mL/100 g) according to the Research Animal Guidelines of Euthanasia

Clinical observation after euthanization: All the lower jaws were photographed and the extraction sockets were detected visually for tissue regeneration.

Sample preparation and staining methods: Mandibles were harvested from the scarified rats and immediately fixed with 10% formalin solution for at least 72 h. Then samples were decalcified in 20% formic acid with a change per week for 10 weeks until decalcification completed. The specimens were dehydrated in ascending grades of ethanol, infiltrated in xylene, embedded in paraffin wax for routine histological processing to obtain tissue paraffin blocks. Blocks of each half jaws were sectioned sagittally into 5 µm thick and then placed on ordinary glass slides and stained with Hematoxylin and Eosin (H and E) staining. Masson's trichrome (MT) staining was also used to detect osteoid tissue and collagen fibers in the examined sections. Also, four micrometer sections were mounted on OptiPlus™ positive-charged microscope slides and subjected to anti-osteopontin antibody immunohistochemical staining. Anti-osteopontin antibody is mouse polyclonal IgG antibody that was purchased from Thermo Fisher Scientific Company, UK (Cat. # RB-9097-R7 (7.0 mL)) and prediluted (ready-to-use) for immunohistochemical staining using avidin biotin complex method (ABC method).

Histological analysis: By ordinary light microscope, histological analysis was performed by a pathologist in a blinded fashion to detect the histopathological bone changes in the H and E stained sections and to evaluate the histochemical reaction of MT within the examined tissues. Histologic parameters were evaluated in extraction sockets and adjacent alveolar bone, as previously reported^{8,11,20,23}. Histologic interpretations were done as follows:

- The presence of osteonecrosis or necrotic foci, defined as presence of eight to ten adjacent empty lacunae in the alveolar bone and presence of osteoclasts or areas of resorption
- Presence and distribution of inflammatory cell infiltrate
- Alveolar bone remodeling, new bone organization and trabecular features and vascularization
- By the computer image analyzing, the histochemical and immunohistochemical stained sections were examined and measured as an area percentage of stained areas and calculated per field at (400X) magnification by image analyzer computer system using the software Leica Qwin 500 (Germany). Areas of the most intense staining were selected then the computer system converted the picture into a blue binary color that could be measured. Five entire fields for each section were measured by experienced oral pathologist in a blinded fashion. Mean values of area percent were obtained for each group for statistical evaluation

Statistical analysis: The area percent values of histochemical and immunohistochemical stained sections obtained from the computer image analysis were statistically described in terms of mean $\pm$ standard deviation ($\pm$ SD) by using a SPSS statistical package (Version 19, Chicago, IL, USA). Student t-test was performed to detect significance between two groups. Results were expressed in the form of p-value, level of significance was set at $p > 0.05$ for all statistical analysis.

RESULTS

Clinical findings: The hallmarks of osteonecrosis of the jaw were visually detected intra-orally after 3 weeks of teeth extraction as inflamed unhealed extraction sockets, no soft tissue coverage with presence of white to opaque exposed bone at the site of extraction. After MSCs application and rats euthenization, signs of healed socket regeneration were observed in MSCs group. These signs were presented as soft tissue regeneration over the sockets with variable epithelial thickening and continuity. Gradual improvement from 2 till 4 weeks intervals were observed. Whereas in control group, exposed necrotic sockets with inflamed soft tissue around remained at 2 and 4 weeks intervals (Fig. 3, 4).

Histological results: At 2 weeks interval, osteonecrotic features were detected in tissue sections of control group as empty osteocytic lacunae, absence of vascularity within bone and irregular resorptive bone surfaces (Fig. 5). Whereas, tissue



Fig. 3: Photograph of lower first molar alveolar socket in control group at 2 weeks interval exhibited inflamed unhealed socket (circle frame)



Fig. 4: Photograph of lower first molar alveolar socket in MSCs group at 2 weeks interval presented with partial epithelial coverage on the alveolar socket, with no signs of inflammation (circle frame)

sections of MSCs group at 2 weeks interval showed signs of bone healing in most of cases detected as presence of abundant mesenchymal cells around bone sequester. Bone remodeling was shown in form of extensive osteoid deposition with osteoblastic proliferation associated with increased vascularity (Fig. 6). At 4 weeks interval in control group, the necrotic bone revealed similar results of 2 weeks interval group with more extensive empty lacunae without osteocytes within irregular thin and thick bone trabeculae enclosing hyalinized bone marrow spaces. The tissue sections of MSCs group showed more organized tissue maturation with good evidence of vascularity

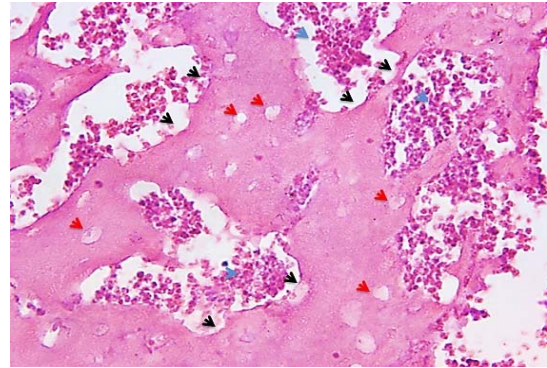


Fig. 5: Photomicrograph of control group at 2 weeks interval showing osteonecrotic bone with empty osteocytic lacunae (red arrows), mixed inflammatory cellular infiltration (blue arrows) and numerous resorption areas (black arrows)

H and E X200

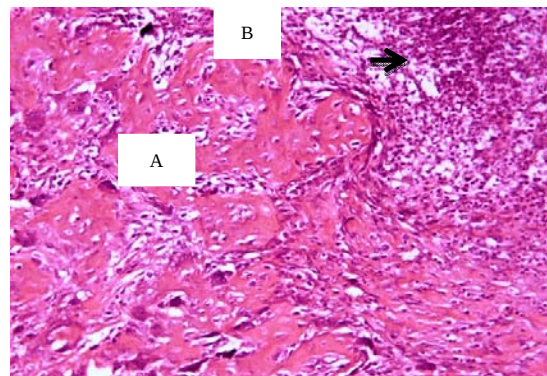


Fig. 6: Photomicrograph of MSCs group at 2 weeks interval showing extensive osteoid deposition (A) with osteoblastic proliferation (B) and diffuse mixed inflammatory cell infiltrate (black arrows)

H and E X200

and bone healing. Area of mature trabeculae with osteocytes and active abundant mesenchyme were also present.

Histochemical results: Masson trichome stained specimens in control group at 2 weeks interval, revealed blue reactions in necrotic bone and granulation tissue (Fig. 7). The MSCs treated specimens at 2 weeks interval showed a wider area of blue colour denoting newly formed collagen fibers and osteoid tissue (Fig. 8).

At 4 weeks interval in control group, necrotic bone and new collagenous stroma with minimal bone formation were presented. The MSCs treated specimens at 4 weeks interval

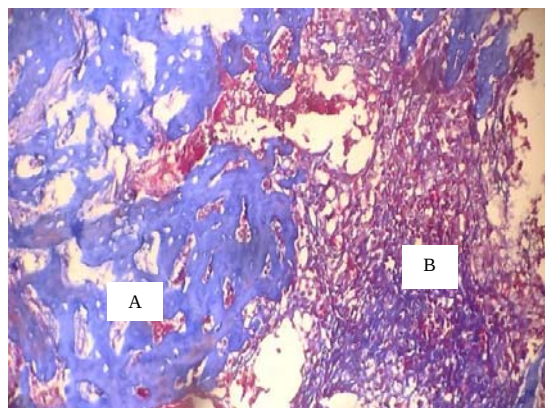


Fig. 7: Photomicrograph of control group at 2 weeks interval showing necrotic bone (A) and granulation tissue (B)
MT X200

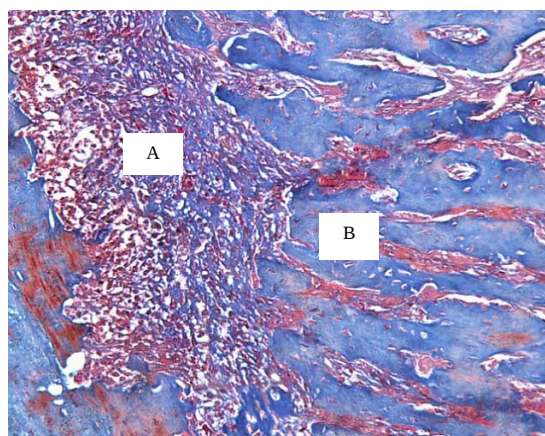


Fig. 8: Photomicrograph of MSCs group at 2 weeks interval showing wider area of new collagen (A) and bone formation (B)
MT X200

Table 1: Comparison between mean values of control and MSCs groups in Masson's trichrome stain

Sacrifice date	Group	Mean	SD	p-value
2 weeks	Control	17.98	1.43	<0.0001
	MCSs	26.50	2.04	
4 weeks	Control	15.76	2.03	<0.0001
	MSCs	35.45	1.89	

SD: Standard deviation, p-value<0.01 highly significant

showed wide area of collagen formation and numerous dilated blood vessels and an intense blue stain of newly formed collagen fibers with well-organized new bone trabeculae with central calcification (Fig. 9). High statistical significant increase ($p<0.0001$) in the mean values of area percent of newly formed collagen and osteoid tissue at 2 and 4 weeks was reported in MSCs group in comparison to those in control group (Table 1).

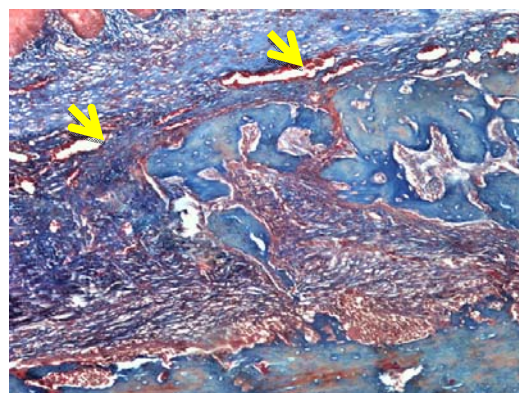


Fig. 9: Photomicrograph of MSCs group at 4 weeks showing wide area of collagen formation and numerous dilated blood vessels (yellow arrows)
MT X100

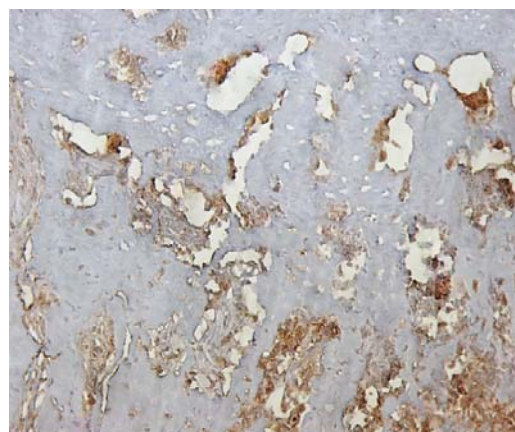


Fig. 10: Photomicrograph of control group at 2 weeks interval showing +ve OPN expression in bone marrows spaces and collagenous stroma and -ve OPN expression in bone cells within necrotic bone
Anti-OPN antibody X200

Immunohistochemical results: The expression of osteopontin was decreased from 2-4 weeks intervals of control group. Osteopontin (OPN) stain was detected sparsely in endosteal cells and cells along the bone-periosteal transitional zone. The number of OPN expressing cells was reduced in necrotic bone (Fig. 10). In MSCs group, the osteopontin expression increased from 2-4 weeks intervals. The positive immunoexpression of OPN was detected throughout the entire bone sections in MSCs group, more intense at endosteal and bone marrows spaces (Fig. 11). High statistical significant increase ($p<0.0001$) in the mean value of area percentage of osteopontin expression at 2 and 4 weeks intervals in MSCs group was reported in comparison to those in control group (Table 2).

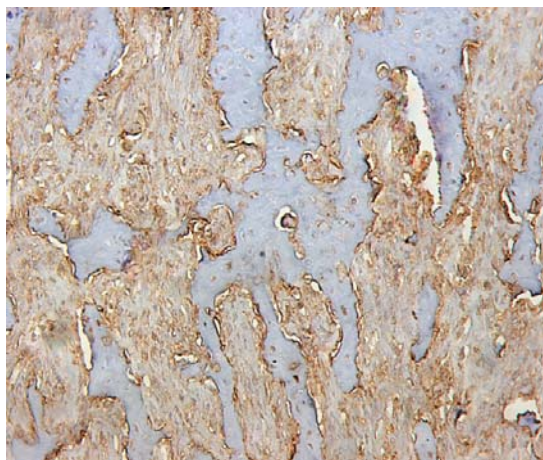


Fig. 11: Photomicrograph of MSCs group at 2 weeks interval showing intense +ve OPN expression in collagenous stroma and bone cells
Anti-OPN antibody X200

Table 2: Comparison between mean values of control and MSCs groups in osteopontin immunostaining

Sacrifice date	Group	Mean	SD	p-value
2 weeks	Control	2.27	0.191	<0.0001
	MSCs	7.22	0.38	
4 weeks	Control	1.19	0.24	<0.0001
	MSCs	12.41	0.43	

SD: Standard deviation, p-value<0.01 highly significant

DISCUSSION

In the present study, the effect of topical application of MSCs on the healing (regenerative) potentials of the bone in bisphosphonate-induced osteonecrotic teeth sockets in rat model, rodents such as mice and rat have been the animal of choice as a model for most human diseases, because of their ease of care and handling, high reproductive capacity, completed genome mapping that further facilitates genetic engineering to suit the disease to be studied and above all, a genetic similarity with humans^{24,25}.

In the present study, the administration of BPs combined with dexamethasone increased the incidence of BRONJ-like models which is clinically relevant because most cancer patients receive multiple immunosuppressive drugs, including dexamethasone and chemotherapeutic agents, suggesting that immunosuppressive therapy may render rat more susceptible to BRONJ lesions development^{8,18,20,26}.

Based on that dentoalveolar surgery, which is a significant risk factor for development of BRONJ, the dental extraction procedures were performed in the current study to increase the susceptibility of developing BRONJ and to simulate the real conditions that can lead to BRONJ^{20,26}. This type of bone necrosis may be related to the impairment of bone capacity to

accommodate the increasing demand for healing that is required in situations of tissue trauma such as tooth extraction²³.

Although recent studies suggested that intravenous (I.V.) systemic application of MSCs was effective for treating BRONJ-like animal models through their immunomodulation properties⁸. However, this application method might do not engraft MSCs to the diseased area and circulate throughout the body, resulting in pulmonary embolism and even death in some experimental animals²⁷. Moreover, MSCs I.V. may provide a tumor microenvironment by generating cytokine networks that promotes the proliferation of cancer cells or accelerate human tumor growth in BRONJ patients who had cancer¹². Therefore, local administration of MSCs may be safe than a systemic injection and allows the benefits of MSCs to be concentrated in the area of interest^{11,12}.

The current study focused on the changes that occurred at the 2nd week interval as well as late changes at 4th week interval after bone marrow derived MSCs application⁸. The histological results of this study showed a delay of healing in the control group compared with MSCs group at 2 and 4 weeks intervals. The delay in initial socket healing seems to be secondary in disruption of the bone remodeling mechanism, allowing the persistence of defective and necrotic bone which was found adjacent to areas of intense local inflammatory infiltrates, suggesting an association between inflammation and tissue degeneration/necrosis in BRONJ-like disease. This was in agreement with other previous studies of^{8,20,26}.

This study considering that BRONJ lesions could be a result of ischemic changes to tissues, researchers observational result demonstrated an important impairment of vascularization in the later stages of alveolar healing in control group at 2 and 4 weeks interval, respectively. These findings can be reasonably explained by the inhibitory effects of BPs on angiogenesis²⁸.

Regeneration signs in MSCs group at 2 and 4 weeks intervals may be attributed to the osteoblastic differentiation of MSCs via a process termed intramembranous ossification in necrotic bone environment^{29,30}. Furthermore, the immunomodulatory, anti-inflammatory and anti-apoptotic paracrine effects of MSCs might have an angiogenic activation responsible for mesenchymal proliferation^{8,10,13}. The MSCs may stimulate angiogenesis by secreting Vascular Endothelial Growth Factor (VEGF) and Hepatocyte Growth Factor (HGF) and stabilize new blood vessels by differentiating into the pericyte phenotype^{31,32}. This was consistent with present study findings where new capillary sprouts were found within the provisional matrix at 2 weeks and more maturation observed at 4 weeks interval in MSCs group¹².

Histochemical results with Masson's trichrome stain at 2 and 4 weeks intervals, revealed significant decrease in collagen and osteoid bone formation in control group compared with MSCs group, indicating marked new bone formation in MSCs group.

Osteopontin is extracellular protein regulates many pathological and physiological processes, including tissue repair, inflammation, fibrosis, biomineralization and immune regulation^{33,34}. In the present study, significant decrease of osteopontin expression was detected in BRONJ lesions of control group compared with MSCs group. Since the effect of MSCs on osteogenesis are well-established in the literature^{14,15}, the current finding might attributed to a continuous state of bone turnover by MSCs differentiation and proliferation³⁵.

CONCLUSION

It is concluded that MSCs topical treatment approach might help in restoring, maintaining or improving defective tissue functions that have been compromised by BRONJ and can potentially offer a safe and effective therapeutic modality for treating BRONJ.

ACKNOWLEDGMENT

Authors are grateful for all the staff of the Research Unit, Faculty of Oral and Dental Medicine, Cairo University for the facilities they offered during the course of this study.

REFERENCES

- Marx, R.E., 2003. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: A growing epidemic. *J. Oral Maxillofacial Surg.*, 61: 1115-1117.
- Ruggiero, S.L., T.B. Dodson, L.A. Assael, R. Landesberg, R.E. Marx and B. Mehrotra, 2009. American Association of Oral and Maxillofacial surgeons position paper on bisphosphonate-related osteonecrosis of the jaws-2009 update. *J. Oral Maxillofacial Surg.*, 67: 2-12.
- Saad, F., J.E. Brown, C. Van Poznak, T. Ibrahim and S.M. Stemmer *et al.*, 2011. Incidence, risk factors and outcomes of osteonecrosis of the jaw: Integrated analysis from three blinded active-controlled phase III trials in cancer patients with bone metastases. *Ann. Oncol.*, 23: 1341-1347.
- Marx, R.E., Y. Sawatari, M. Fortin and V. Broumand, 2005. Bisphosphonate-induced exposed bone (osteonecrosis/osteopetrosis) of the jaws: Risk factors, recognition, prevention and treatment. *J. Oral Maxillofac. Surg.*, 63: 1567-1575.
- Parekkadan, B. and J.M. Milwid, 2010. Mesenchymal stem cells as therapeutics. *Ann. Rev. Biomed. Eng.*, 12: 87-117.
- Salamon, A. and E. Toldy, 2009. Use of mesenchymal stem cells from adult bone marrow for injured tissue repair. *Orvosi Hetilap*, 150: 1259-1265.
- Leventhal, A., G. Chen, A. Negro and M. Boehm, 2012. The benefits and risks of stem cell technology. *Oral Dis.*, 18: 217-222.
- Kikuri, T., I. Kim, T. Yamaza, K. Akiyama and Q. Zhang *et al.*, 2010. Cell based immunotherapy with mesenchymal stem cells cures bisphosphonate related osteonecrosis of the jaw-like disease in mice. *J. Bone Mineral Res.*, 25: 1668-1679.
- Li, Y., J. Xu, L. Mao, Y. Liu and R. Gao *et al.*, 2013. Allogeneic mesenchymal stem cell therapy for bisphosphonate-related jaw osteonecrosis in Swine. *Stem Cells Dev.*, 22: 2047-2056.
- Ogata, K., W. Katagiri, M. Osugi, T. Kawai and Y. Sugimura *et al.*, 2015. Evaluation of the therapeutic effects of conditioned media from mesenchymal stem cells in a rat bisphosphonate-related osteonecrosis of the jaw-like model. *Bone*, 74: 95-105.
- Barba-Recreo, P., T. Georgiev-Hristov, E.R. Bravo-Burguillos, A. Abarrategi, M. Burgueno and M. Garcia-Arranz, 2015. Adipose-derived stem cells and platelet-rich plasma for preventive treatment of bisphosphonate-related osteonecrosis of the jaw in a murine model. *J. Cranio-Maxillo-Facial Surg.*, 43: 1161-1168.
- Kaibuchi, N., T. Iwata, M. Yamato, T. Okano and T. Ando, 2016. Multipotent mesenchymal stromal cell sheet therapy for bisphosphonate-related osteonecrosis of the jaw in a rat model. *Acta Biomaterialia*, 42: 400-410.
- Matsuura, Y., I. Atsuta, Y. Ayukawa, T. Yamaza and R. Kondo *et al.*, 2016. Therapeutic interactions between mesenchymal stem cells for healing medication-related osteonecrosis of the jaw. *Stem Cell Res. Ther.*, Vol. 7. 10.1186/s13287-016-0367-3.
- Cella, L., A. Oppici, M. Arbasi, M. Moretto and M. Piepoli *et al.*, 2011. Autologous bone marrow stem cell intralesional transplantation repairing bisphosphonate related osteonecrosis of the jaw. *Head Face Med.*, Vol. 7. 10.1186/1746-160X-7-16.
- Gonzalez-Garcia, M., F.J. Rodriguez-Lozano, V. Villanueva, D. Segarra-Fenoll and M.A. Rodriguez-Gonzalez *et al.*, 2013. Cell therapy in bisphosphonate-related osteonecrosis of the jaw. *J. Craniofacial Surg.*, 24: e226-e228.
- Allen, M.R. and D.B. Burr, 2009. The pathogenesis of bisphosphonate-related osteonecrosis of the jaw: So many hypotheses, so few data. *J. Oral Maxillofacial Surg.*, 67: 61-70.
- Voss, P.J., M. Stoddart, T. Ziebart, S. Zeiter and K. Nelson *et al.*, 2015. Zoledronate induces osteonecrosis of the jaw in sheep. *J. Cranio-Maxillo-Facial Surg.*, 43: 1133-1138.
- Hokugo, A., R. Christensen, E.M. Chung, E.C. Sung and A.L. Felsenfeld *et al.*, 2010. Increased prevalence of bisphosphonate related osteonecrosis of the jaw with vitamin D deficiency in rats. *J. Bone Mineral Res.*, 25: 1337-1349.

19. Zecchin, K.G. and J. Jorge, 2007. A new method for extraction of mandibular first molars in rats. *Brazil. J. Oral Sci.*, 6: 1344-1348.
20. Sonis, S.T., B.A. Watkins, G.D. Lyng, M.A. Lerman and K.C. Anderson, 2009. Bony changes in the jaws of rats treated with zoledronic acid and dexamethasone before dental extractions mimic bisphosphonate-related osteonecrosis in cancer patients. *Oral Oncol.*, 45: 164-172.
21. Maahs, M.P., A.A. Azambuja, M.M. Campos, F.G. Salum and K. Cherubini, 2011. Association between bisphosphonates and jaw osteonecrosis: A study in Wistar rats. *Head Neck*, 33: 199-207.
22. Alhadlaq, A. and J.J. Mao, 2004. Mesenchymal stem cells: Isolation and therapeutics. *Stem Cell. Dev.*, 13: 436-448.
23. Conte Neto, N., L.C. Spolidorio, C.R. Andrade, A.S. Bastos, M. Guimaraes and E. Marcantonio, 2013. Experimental development of bisphosphonate related osteonecrosis of the jaws in rodents. *Int. J. Exp. Pathol.*, 94: 65-73.
24. Sharma, D., S. Hamlet, E. Petcu and S. Ivanovski, 2013. Animal models for bisphosphonate related osteonecrosis of the jaws An appraisal. *Oral Dis.*, 19: 747-754.
25. Barba-Recreo, P., M. Garcia-Arranz, L. Yebenes and M. Burgueno, 2014. Zoledronic acid-related osteonecrosis of the jaws. Experimental model with dental extractions in rats. *J. Cranio-Maxillo-Facial Surg.*, 42: 744-750.
26. Abtahi, J., F. Agholme and P. Aspenberg, 2013. Prevention of osteonecrosis of the jaw by mucoperiosteal coverage in a rat model. *Int. J. Oral Maxillofacial Surg.*, 42: 632-636.
27. Tatsumi, K., K. Ohashi, Y. Matsubara, A. Kohori and T. Ohno *et al.*, 2013. Tissue factor triggers procoagulation in transplanted mesenchymal stem cells leading to thromboembolism. *Biochem. Biophys. Res. Commun.*, 431: 203-209.
28. Kobayashi, Y., T. Hiraga, A. Ueda, L. Wang and M. Matsumoto-Nakano *et al.*, 2010. Zoledronic acid delays wound healing of the tooth extraction socket, inhibits oral epithelial cell migration and promotes proliferation and adhesion to hydroxyapatite of oral bacteria, without causing osteonecrosis of the jaw, in mice. *J. Bone Mineral Metabol.*, 28: 165-175.
29. Salem, H.K. and C. Thiemermann, 2010. Mesenchymal stromal cells: Current understanding and clinical status. *Stem Cells*, 28: 585-596.
30. Knight, M.N. and K.D. Hankenson, 2013. Mesenchymal stem cells in bone regeneration. *Adv. Wound Care*, 2: 306-316.
31. Murphy, M.B., K. Moncivais and A.I. Caplan, 2013. Mesenchymal stem cells: Environmentally responsive therapeutics for regenerative medicine. *Exp. Mol. Med.*, Vol. 45. 10.1038/emm.2013.94.
32. Shao, J., W. Zhang and T. Yang, 2015. Using mesenchymal stem cells as a therapy for bone regeneration and repairing. *Biol. Res.*, Vol. 48 10.1186/s40659-015-0053-4.
33. Shaheen, M. and N.L. Weintraub, 2007. Osteopontin: A bona fide mediator of abdominal aortic aneurysm? *Arterioscler. Thromb. Vasc. Biol.*, 27: 439-441.
34. Pagel, C. N., D.K.W. Wijesinghe, N.T. Esfandouni and E.J. Mackie, 2014. Osteopontin, inflammation and myogenesis: Influencing regeneration, fibrosis and size of skeletal muscle. *J. Cell Commun. Signal.*, 8: 95-103.
35. Wang, C., H. Meng, X. Wang, C. Zhao, J. Peng and Y. Wang, 2016. Differentiation of bone marrow mesenchymal stem cells in osteoblasts and adipocytes and its role in treatment of osteoporosis. *Med. Sci. Monitor: Int. Med. J. Experiment. Clin. Res.*, 22: 226-233.