Saudi Dental Journal (2022) 34, 100–106

King Saud University

Saudi Dental Journal

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ORIGINAL ARTICLE

Treatment of bisphosphonate induced osteonecrosis of jaw in rats using an angiogenesis factor (A-Heal) and ABMDO (Autologous Bone Marrow Derived Osteoblasts)

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Received 29 August 2021; revised 6 December 2021; accepted 15 December 2021
Available online 23 December 2021

KEYWORDS

Bisphosphonate-associated osteonecrosis of the jaw;
Osteoblasts;
Angiogenesis factor;
Osteoporosis;
Bisphosphonates;
Stem cell

Abstract *Background and objective:* The aims of this study were to create Bisphonates Related Osteonecrosis of the Jaw (BRONJ) in rats and treat them with an angiogenesis factor (A-Heal) and ABMDO (Autologous Bone Marrow Derived Osteoblasts).

Materials and methods: Thirty female Wistar rats were procured. Rats were labeled as Group I to III. Group I = Osteoblast group, Group II = A-Heal and Group III Control group. In Groups I-III, BRONJ was created and treated in Group I with ABMDO, Group II with A-Heal and Group III was the control group. At the end of the four weeks post treatment, all the animals were humanely killed. The intact maxillae were removed in total. Histopathological and radiological examinations were carried out with physicians blinded to the groups.

Results: Computerized tomography revealed that Groups I and II demonstrated the presence of dense osteosclerosis, intralesional calcifications, and adequate healing of the overlying soft tissues compared to Group III, which showed the presence of bone erosions at the alveolar ridge with a lack of intralesional calcifications and ulceration of the overlying soft tissues. Histologically,

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sdentj.2021.12.006

H&E staining Group 1 and Group 2 both showed marked reactive bone formation. Group 2 additionally revealed the most prominent vascular proliferation (also highlighted by Factor VIII, an endothelial cell marker) among all groups. Group 3 showed cartilaginous proliferation with less reactive bone formation, implicating decreased endochondral ossification compared to Groups 1 and 2.

Conclusion: This study shows that angiogenesis factor (A-Heal) and ABMDO were successful in the treatment of experimentally created BRONJ in an animal model.

1. Introduction

Bisphosphonates are a group of drugs that prevent bone loss and hence are used in the management of osteoporosis. In the recent past, bisphosphonates became the first-line drug in the treatment of postmenopausal osteoporosis (Watts et al., 2010; North American Menopause Society, 2010; Hauk, 2013). Osteonecrosis of jaw (ONJ) was sporadically reported in patients undergoing treatment for cancer with concomitant steroid therapy. With bisphosphonates as first-line treatment for osteoporosis, the numbers of ONJ have increased and come into focus. ONJ affects the maxilla or the mandible and is associated with bisphosphonate therapy, and in 2003, the condition was defined as a pathological entity (Compston et al., 2013). Many theories have been proposed for the cause of ONJ, and it was suggested that ONJ occurs primarily due to disruption of vascular supply followed by impaired angiogenesis (Wan et al., 2020; Mücke et al., 2016; Pazianas, 2011; Reid, 2009). There is no gold standard treatment recommended for BRONJ, and many treatments have been suggested, but the outcome of ONJ remains protracted and grim. There is no specific treatment, and studies on nonsurgical therapeutic modalities could improve the prognosis and reduce the morbidity of ONJ. Since one treatment was not successful, a combination of antibiotics and corticosteroids, hyperbaric oxygenation (HBO), bone debridement, and surgical resection followed by reconstruction was attempted. (Zarychanski et al., 2006; Abu-Id et al., 2006; Marx 1983) Nevertheless, an effective treatment for bisphosphonate-associated ONJ remain elusive and further options need to be studied (Ferreira et al., 2021; McLeod and Bater, 2010). Bisphosphonate-related osteonecrosis of the jaw in rats has been created so that newer and more effective treatment of ONJ can be developed (Nagai et al., 2007; Woo et al. 2006). Recently, an animal study showed that local application of fluvastatin successfully treated animals with related osteonecrosis of the jaw (Sanda et al., 2021). The two modalities that we are using in this study are also topical applications at the site of ONJ. The objective of this study was to create ONJ using intravenous bisphosphonates and treat it with ABMDO and A-Heal (Sadat-Habdan Mesenchymal Stimulating peptide), an angiogenesis factor.

2. Materials and methods

Jang et al. (2015) showed that rats treated with bisphosphonates and steroids are the most reliable and reproducible animals for developing BRONJ; hence, in this study, a rat model was used. Before starting the study, approval of the Institutional Review Board was obtained. Thirty female Wistar rats aged $\geq$3 months and weighing $\geq$300 g were procured. A minimum of 10 rats in each group was chosen to give the power of analysis as per the calculation of Charan et al. (2013). Rats were labeled as Group I to III. Group I = Osteoblast group, Group II = A-Heal, and Group III Control. All rats in Groups I to III were given 80 μg/kg intravenous zoledronate once a week for 4 weeks. After 21 days, maxillary first molars were removed. At the time of tooth extraction, bone marrow was aspirated from the iliac crest to isolate MSCs and osteoblasts as previously described (Piao et al., 2005). Prior to treatment, two animals from Groups I, II, and III were euthanized to confirm the development of BRONJ by radiology and histopathology. At 12 weeks of tooth extraction, rats in Group I had 2 million osteoblasts in 0.5 ml injected at the site of BRONJ and in group II, 5 mg/kg body weight of angiogenesis factor was put directly at the site three times a week for three weeks and in group III saline was injected at the site of extraction. At the end of the four weeks, all the animals were humanely killed. The maxillas were removed in total. Histologically, the presence of osteoclasts, presence of exposed bone, inflammatory infiltration and integrity of epithelial tissue with development of many empty bone lacunae, marginal bone loss, necrotic bones with inflammatory cells and ulcer formation were observed. A computerized scan was performed to compare the radiological findings related to bone loss, rarefaction, osteolysis, and healing. A Somatcom Definition Flash machine was used with 120 kV, MAS 95, scan time 14.8 s and 128 (0.4 mm cuts) slices. The entire study was performed at the Animal Lab of Institute of Research and Medical Consultations of Imam AbdulRahman Bin Faisal University, Dammam.

3. Results

All animals withstood the procedure well and no deaths occurred until euthanization. Post injection of the 80 μg/kg intravenous zoledronate 80 μg/kg and at 12 weeks of tooth extraction, 2 animals from each group were euthanized and the intact maxillas were harvested en bloc and BRONJ was confirmed in the animals by radiological and histological analysis.

In group I and II, the soft tissue had healed with the closure of the overlying soft tissue where as in group III there was mucositis and ulceration with area of necrosis. Fig. 1 shows gross pictures of the three groups. Fig. 2 reveals the computerized tomography of the three groups. In the treated groups, there was visible a thickening of trabecular bone, and apposi-

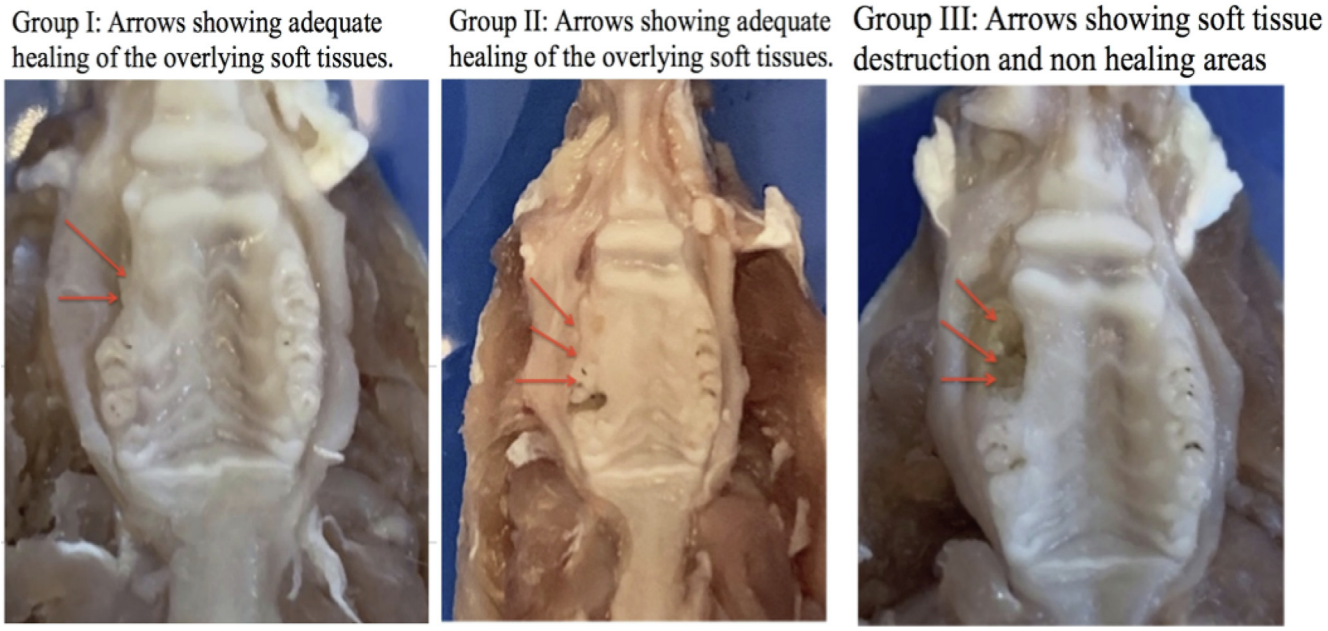

Fig. 1 shows gross pictures of the three groups. Arrows point to the area of extraction. In group I and II there was complete healing of the soft tissues while in group III the area of necrosis of visible.

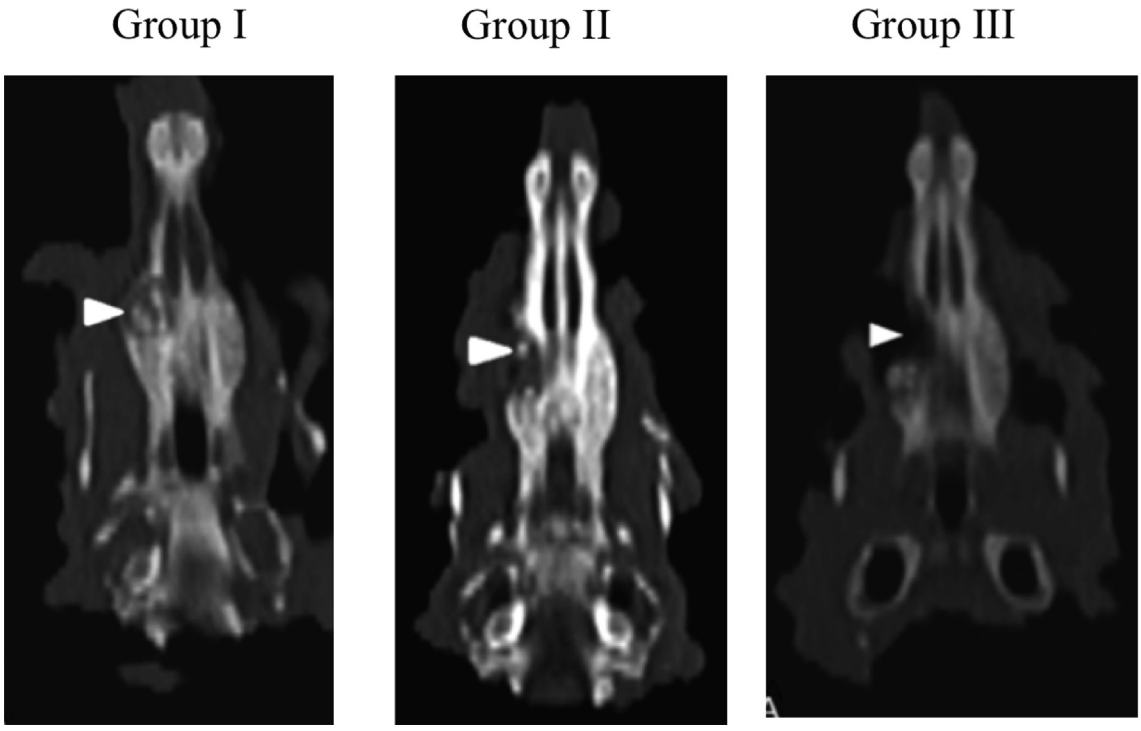

Fig. 2 reveals the computerized tomography of the three groups. In the treated groups the images show startling changes of healing by way osteoclerosis, calcifications and the small intralesional calcifications. In the control group III the presence of bone erosions at the alveolar ridges with minimal osteosclerosis and lack of intralesional calcifications all suggestive of lack of healing process.

tional growth of osseous tissue at endosteal surfaces. In control group III, the presence of bone erosions at the alveolar ridges with minimal osteosclerosis and lack of calcium deposition in the area of extraction were all suggestive of a lack of healing.

Histological findings as seen in the routine H&E stain are depicted in Figs. 3 and 4. Fig. 3 shows prominent reactive bone formation beneath the surface in the treated groups I and II, while control group III showed less reactive bone formation. Inflammatory changes were pronounced in Group III that also

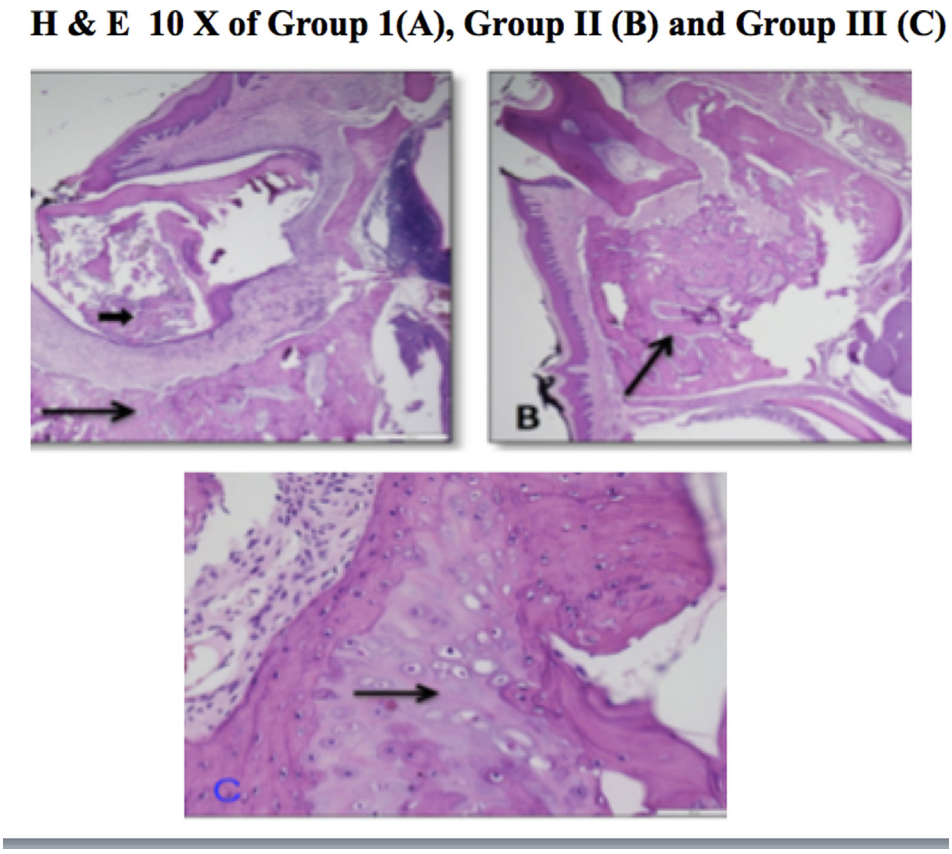

Fig. 3 shows Histologically H & E staining of the groups showed reactive new bone formation while the control group III showed fibrosis with minimal to the reactive bone and attempt to heal. Group III showed severe inflammation and fibrosis as compared to other groups and also showed exaggerated proliferation of cartilage (arrow) within the limited reactive bone. (H&E; 40x).

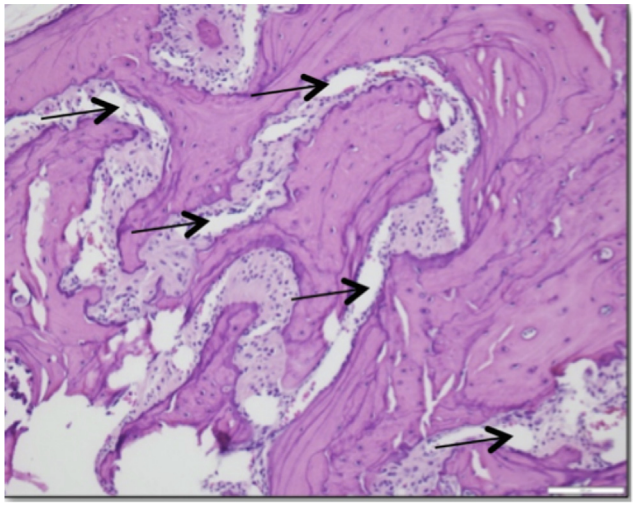

Fig. 4 is the section of Group II animals in which angiogenesis factor was used which showed exuberant vascular proliferation.

showed fibrosis with exaggerated proliferation of cartilaginous tissue along with the limited reactive bone formation, implying limited endochondral ossification or healing. Fig. 4 shows exuberant vascular proliferation in Group II animals, which were administered the angiogenesis factor.

Immunochemical staining for evaluation of the Ki-67 proliferation index showed that Groups I and II (Fig. 5, A and B) had similar moderate proliferation indices within and adjacent to the reactive bone. Groups III showed minimal proliferation index. (Fig. 5, C). Factor VIII, an endothelial marker was used to assess extent of vascular proliferation, and Group 2 (Fig. 6, B) showed the most prominent vascular proliferation.

4. Discussion

Our study shows that BRONJ can be treated with angiogenesis factors and ABMDOs in a rat model. Healing by angiogenesis factors was superior to ABMDO when compared to the control group. The antiangiogenic action of bisphosphonates has been reported in experimental animals (Nagai et al., 2007). Added to this concept, the capability of bisphosphonates to exhibit antiangiogenic actions was shown by decreasing VEGF levels. Indirectly, angiogenesis factors could be an important therapeutic modality, which can be exploited to reduce the morbidity of patients with BRONJ.

Many causes have been implicated in the development of BRONJ from infection (Aspenberg 2006; Dodson et al., 2008). Osteonecrosis through effects on blood vessels in bone through inhibition of vascular endothelial growth (Santini et al., 2002), and reduced resorptive activity due to bisphosphonates main action effect diminished healing of the lesions.

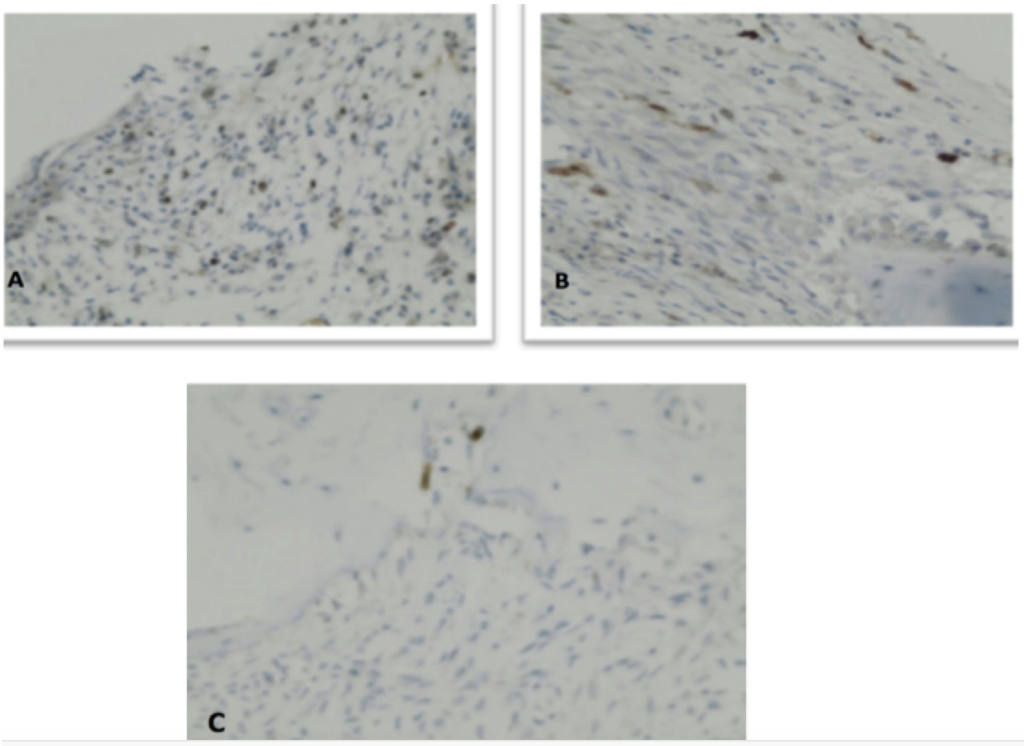

Fig. 5 Immunochemistry staining using Ki-i67 proliferation index showed that Groups I and II (A and B) showed similar moderate proliferation indices within and adjacent to the reactive bone. Groups III (C) showed minimal proliferation. (Ki67 IHC; nuclear stain; 40x).

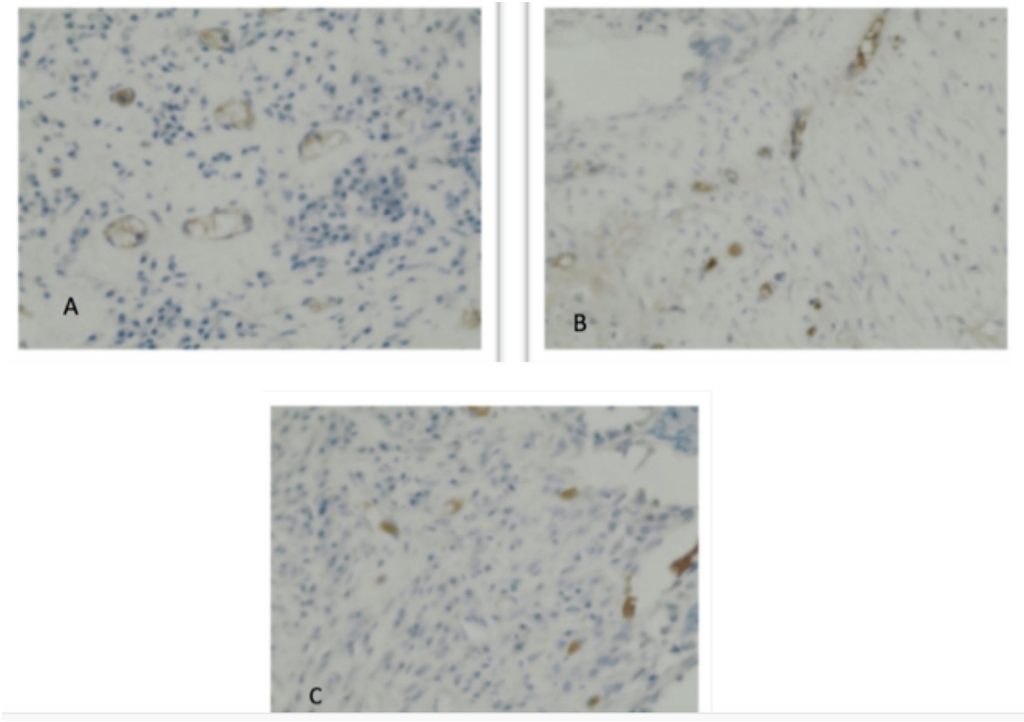

Fig. 6 Sections with staining with Factor VIII endothelial marker was used to assess vascular proliferation and Group 2 (B) show the most prominent vascular proliferation highlighted by Factor VIII staining.

Based on these assumptions, treatments were instituted from local to systemic antibiotics and surgical excision (Ruggiero et al., 2004; Melo and Obeid, 2005; Wilde et al., 2011). Barba-Recreo et al. (2015) showed local applications of allogenic adipose tissue-derived stem cells and found that there was an ample reduction in bone necrosis with a substantial increase in bone remodeling in the area of ONJ. Recently, in rat and mouse models, local application of mesenchymal stem cells and platelet-derived growth factor increased angiogenesis and bone healing (Gao et al., 2021; Watanabe et al., 2020; Rollason et al., 2016).

Mozzati et al. (2012) used a combination of surgery and platelet-derived growth factor (PRGF) in 32 cancer patients with BRONJ and indicated that the addition of PRGF to surgery gave better outcomes. Cella et al. (2011), after using autologous bone marrow stem cell transplantation, concluded that treatment with autologous stem cell transplantation resulted in a total and complete recovery of BRONJ. Recently, Bouland et al. (2020), in two patients with BRONJ, topically applied leukocyte-platelet-rich fibrin and stromal vascular fraction and observed soft tissue healing in 14 days followed by new bone formation with no recurrence on follow-up.

Our study has potential limitations in that we did not culture the area to confirm the presence or absence of the infection, which could exacerbate the picture of BRONJ. Second, we removed the maxillary molar rather than the mandible. In conclusion, our study used two different treatment modalities in animals, angiogenesis factor (A-Heal) and ABMDO, and compared with the untreated group, both animals in the treated group showed positive signs of healing and fewer changes in the maxilla at the site of tooth extraction. We believe that this study should be replicated in a larger animal, and if it confirms similar results, then we can move toward Phase I human trials to treat this debilitating, common drug-induced complication.

Funding

The authors sincerely thank the Deanship of Scientific Research at the Imam AbdulRahman Bin Faisal University, Dammam for funding the study video project Number 2019-413.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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