Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Efficacy of Enamel Matrix Derivative on Alveolar Ridge Augmentation by Distraction Osteogenesis

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ARTICLE HISTORY

Received: September 29, 2010
Revised: October 28, 2010
Accepted: October 28, 2010

Key words:
Bone regeneration
Distraction osteogenesis
Enamel matrix derivative

ABSTRACT

Distraction osteogenesis (DO) is a surgical-orthopedic technique for lengthening a bone by separating or distracting a fractured callus. The aim of this study was to observe the effects of an enamel matrix derivative (EMD) on bone repair and regeneration after DO on a canine mandible. Ten adult beagle dogs were used in this study. Their right and left mandibles were compared as the test and control groups, respectively. The distraction was undertaken at a rate of 1 mm per day for 10 consecutive days to yield 10 mm lengthening of the mandibular corpus. The EMD was treated into the test group at the site of the lengthened bone. At 0, 1, 3, 6 and 9 weeks after EMD treatment, the bone mineral density (BMD) at the site of the lengthened bone was measured using quantitative computed tomography. BMD in the tested group was higher during consolidation period than in the control. The difference in the BMD of 1 and 3 weeks after EMD treatment was significant ($p < 0.05$). In histological findings, new bone formation in the test group was denser than the control group. These results suggest that the application of an EMD during DO is suitable method for alveolar ridge augmentation in dogs.

To Cite This Article: Kim JM, JH Kim, TS Han, G Kim, SS Kang and SH Choi, 2011. Efficacy of enamel matrix derivative on alveolar ridge augmentation by distraction osteogenesis. Pak Vet J, 31(2): 145-148.

INTRODUCTION

Alveolar atrophy is a major problem in achieving successful oral rehabilitation using endosseous implants. Recently, bone transplantation (Raghoebar *et al.*, 2010), guided bone regeneration (Lethaus *et al.*, 2010), and/or bone splitting (Gutta and Waite, 2008) in conjunction with the placement of a dental implant have been performed. Distraction osteogenesis (DO) has been applied clinically to augment the alveolar ridge vertically (Zhao *et al.*, 2009). This technique has the advantages of allowing osseous buildup without the need for bone transplantation or simultaneous soft tissue formation. However, this method requires a long treatment period for distraction and proper ossification of the regenerating bone. The consolidation period determines the appropriate time to remove the device. The consolidation period depends on the distraction site, the status of vascularization and the age of the patient (Cho *et al.*, 2004). This protracted treatment extends the period during which the patients are inconvenienced by the presence of a distraction device. Hence, shortening the bony consolidation period would be of great benefit to the patient. A decrease in distraction time while obtaining the optimal properties in the regenerated bone would be desirable.

The enamel matrix proteins and a recently developed enamel matrix derivative (EMD) have been suggested to encourage periodontal tissue regeneration by activating the biosynthesis of cementum, periodontal ligament, and alveolar bone (Kenny, 2009). The EMD appears to enhance the proliferation and total protein production of periodontal ligament cells, as well as to promote mineralized nodule formation (Venezia *et al.*, 2004). In terms of future periodontal ligament tissue bioengineering, an EMD might be an effective biological tool for bone and periodontal tissue regeneration. However, there are few reports showing the effect of an EMD in consolidation during DO. This study evaluated the efficacy of EMD in alveolar ridge augmentation followed by DO in dogs.

MATERIALS AND METHODS

Animals

Ten male beagle dogs (mean age 2 years) in good systemic health, weighing approximately 10kg each, were

used in this study. The experimental animals were divided into two groups. The test group was treated with the EMD after placing the DO device in the right mandible. The control group was treated with a physiological saline solution after placing the DO device in the left mandible. Throughout the study, the animals were given access to a soft diet (PROPLAN®, Nestle Purina Co., Korea) and water *ad libitum*. The Animal Care Committee of Chungbuk National University approved this protocol.

Distraction osteogenesis

All the teeth were scaled and cleaned before DO. The dogs were premedicated with a subcutaneous dose of atropine sulfate (0.04mg/kg, Kwang-Myung Pharm. Co., Korea), and sedated subcutaneously with 2% xylazine (2mg/kg, Rompun®, Bayer, Korea). Anesthesia was induced and maintained with tiletamine and zolazepam (7.5mg/kg, Zoletil®, Virvac, Korea). The left and right mandibular 1st to 4th premolars were extracted without injury using a closed extraction technique and alveoloplasty was performed. After surgery, the mucoperiosteal flap was closely attached without tension using 4-0 single interrupted absorbable sutures. The 1st to 3rd maxillary teeth also were extracted in the same manner in order to prevent ridge trauma while chewing. The DO device was applied 12 weeks after extracting the teeth. The alveolar mucosa was reflected, exposing the lateral surface of the mandible. An osteotomy was applied to the alveolar bone using an oscillating saw and a DO device was then applied. The flaps were repositioned and sutured with 4-0 single interrupted absorbable sutures, and the distraction screw was left protruding from the inferior border of mandible. After all surgical procedures, antibiotics (ampicillin, 20mg/kg, b.i.d, Whanin Pharm Co., Korea) were subcutaneously administered for 6 days to prevent or control any infection, and the oral cavity was rinsed daily with 0.12% chlorhexidine-digluconate during the first two weeks after surgery. The bone edges were maintained in close approximation for 5 days (latency period). The edges were then distracted at a rate of 1mm each day for 10 days (distraction period). This was followed by a 9-week period in which the external fixation was maintained without distraction (consolidation period). Emdogain® (Biora, USA) was used as the EMD. Freeze-dried EMD preparation (30mg) was reconstituted with 1.0ml of a propylene glycol alginate solution according to the operating instructions. On the 3rd day after the distraction, 0.3ml of the EMD and 0.3ml of physiological saline solution were injected into the test (right mandible) and control groups (left mandible), respectively.

Determination of bone mineral density

Computed Tomography (CT) was performed after the distraction, and at 1, 3, 6 and 9 weeks after EMD treatment. High-resolution transverse images of the mandible in dogs were obtained using a conventional CT scanner (Picker IQ, Philips Medical Systems, Netherlands). The CT images were taken contiguously at 1-mm intervals and the hounsfield units in the distracted area were calculated to determine the bone mineral density (BMD).

Histological processing

After 9-week consolidation period, all the dogs were sacrificed, the mandibles were resected *en bloc* and the distracted bone was harvested. The bone was fixed in 10% buffered formalin, decalcified in formic acid, and embedded in paraffin. Five-µm thick sections were cut, stained with hematoxylin-eosin, and observed using optical microscopy.

Statistical analysis

The results for test and control groups are expressed as the mean ± standard deviation, and the data was analyzed using a student's *t*-test. A P value<0.05 was considered significant.

RESULTS

The dogs tolerated the surgical procedures well and showed no discomfort during the distraction period. The overlying alveolar mucosa after completing the distraction had advanced vertically, and the surface and color of the gingiva appeared to be normal. No infection of the surgical area was observed, and the distraction device was well tolerated.

Quantitative CT was used to measure the BMD in the mandible. The serial BMD showed progressive calcification of the distracted zone between the mandibular segments in the two groups. The BMD in the test group was higher during consolidation period than in the control (Table 1). In particular, the difference in the BMD of 1 and 3 weeks during the consolidation period was significant (P<0.05).

The histological findings revealed the formation of woven bone within the distraction gap. In the control group, spindle shaped new bone was observed in the distraction area, and the newly formed bone was arranged parallel to the direction of the distraction (Fig. 1A). Thick new bone formation was observed in the distraction area. Newly formed bone was arranged in a multidirectional manner in the test group (Fig. 1B). The newly formed bone in test group was denser than that of control group.

DISCUSSION

This study focused on bone repair and regeneration for the alveolar ridge augmentation. Horizontal alveolar distraction was successfully performed using a lengthening apparatus that was designed for narrow alveolar ridge animal models. The DO in the deficient alveolar bone has become a challenging method for bone lengthening because of the advantages of osseous build up without the need for a bone transplant. This study compared the new bone formation of the DO after a single injection of EMD to the distracted area. All the dogs were examined for any signs of inflammation, necrosis and wound dehiscence and there were no gross signs found.

DO is a process by which the gradual separation of osteotomized bone edges results in the formation of new bone (Faber *et al.*, 2005). It has been used to improve the skeletal relationship in patients undergoing orthopedic, craniofacial, and maxillary surgery. The indications of DO for reconstructive surgery have been widened, and Zhao *et al.* (2009) demonstrated its application in augmenting the

Table 1: Effect of an enamel matrix derivative (EMD) on bone mineral density (%) after distraction osteogenesis on a canine mandible

Group	After EMD treatment (week)				
	0	1	3	6	9
Test	0	16.56±1.90*	21.60±2.58*	39.82±3.25	67.50±5.10
Control	0	12.58±1.35	15.77±1.88	33.31±1.94	57.80±4.08

All data were expressed as the mean ± SD (n=5). *P<0.05 as compared with control group.

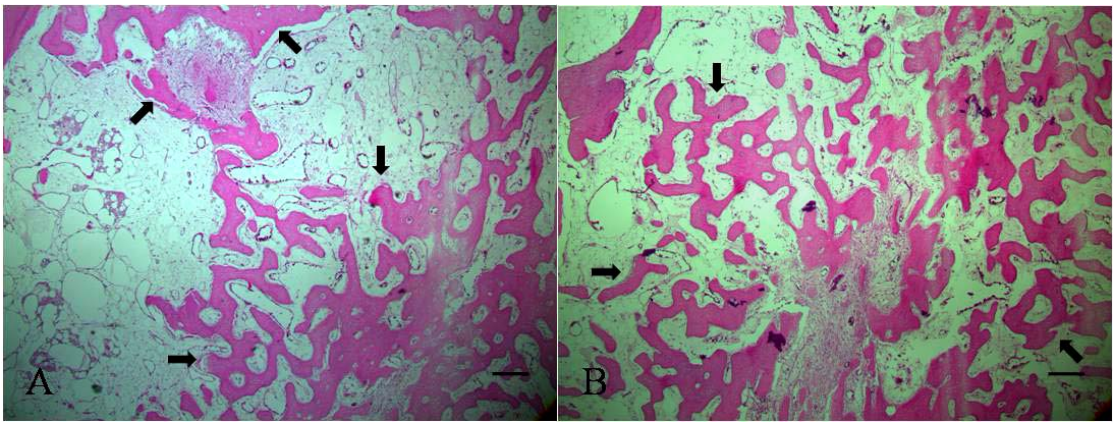

Fig. 1: Histological findings of bone formation within the distraction gap at 9 weeks after enamel matrix derivative treatment. The new bones (arrows) in the test group (B) are denser than that of the control group (A). H&E, × 50, bar = 200 μm.

alveolar bone by distraction. The potential of DO for alveolar ridge augmentation has been described using animal experiments in many manuscripts (Bavitz *et al.*, 2000; Nosaka *et al.*, 2002), which has improved the understanding of the anatomical, topographical and physiological properties of DO in dogs. The process and the clinical application of DO were studied extensively, and it was reported that an increase in fixator stability enhanced the level of bone formation (Ilizarov, 1989). In our study, the screw of the DO device was left protruding from an inferior border of the mandible in order to prevent disturbances in bone distraction as well as damage to the tongue during movement. By demonstrating the initial areas of mineralization at the beginning of the consolidation period, the results indicate that new bone formation during alveolar osteodistraction begins before the 14th day of distraction from the initial areas of mineralization at the beginning of the consolidation period. This timeframe is within the range for limb lengthening (7 to 14 days) that was previously reported (Cope and Samchukov, 2000). The BMD of the test group increased with time and was 67.5% at 9 weeks after EMD treatment. It is believed that the fixation in our study would be sufficiently rigid to achieve the desired result.

The aim of DO is to obtain optimal bone lengthening (Mizumoto *et al.*, 2003). Despite the use of an appropriate distraction rate, the formation of new bone is not always optimal. There have been several studies aimed enhancing the level of bone formation and maturation, and thereby shortening the treatment time (Matsuyama *et al.*, 2005). This study focused on the consolidation period that could shorten the DO process. An EMD has been reported to stimulate periodontal tissue regeneration through the activation of cementum biosynthesis, periodontal ligament and alveolar bone (Sculean *et al.*, 2007). Although an EMD has already been used clinically, the mechanisms by which it promotes *in situ* bone formation are unclear. Kawana *et al.* (2001) reported that an EMD had an osteopromotive effect on bone and assisted in medullary regeneration during wound healing of injured long bones. An EMD was also reported to increase the initial ingrowth of bone trabeculae around endosseous implants through new bone induction in the marrow cavities and maintained such bony support of implants by fulfilling the implant surfaces (Shimizu-Ishiura *et al.*, 2002). Suzuki *et al.* (2005) reported that the EMD stimulates the signal transduction of bone morphogenetic protein (BMP) and TGF-β. Recent evidence suggests that BMPs are the key signaling molecules that mediate DO in the enchondral or long bones (Rauch *et al.*, 2000). However, the role of BMPs in DO of the membranous or flat bones is unclear. Fifteen BMPs have been characterized and cloned thus far, and all except for BMP-1 are members of the transforming growth factor β superfamily (Chen *et al.*, 2004). BMPs are multifunctional proteins with a variety of effects on cell growth and differentiation, including osteogenesis (Ai-Aql *et al.*, 2008).

This study examined the radiological and histological evidence of the EMD in bony consolidation during alveolar DO. The radiological results of this experiment suggest that the EMD can increase the level of bony consolidation in DO. The BMD data suggests that the consolidation of the distracted area increased at all time points during the consolidation period. From 1 to 9 weeks of consolidation, consolidation of the distracted area was characterized by a progressive increase in cortical surface. In addition, the consolidation of the test group was higher than the control group. On histological findings, denser new bones were generated in the test group. Newly formed bones are arranged in a multidirectional manner. The application of an EMD on DO for alveolar ridge augmentation increases the rate of new bone formation,

and reduces the consolidation time. In addition, it also allows the earlier removal of the external fixator. These findings suggest that EMD is effective in the early stages of bony consolidation in DO and can shorten the time for implant treatment through the use of DO.

Acknowledgement

This work was supported by a grant (code #20070303034044) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

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