ORIGINAL ARTICLE

Influence of Root Dentin Surface Conditioning with Bone Morphogenetic Protein-2 on Periodontal Wound Healing in Beagle Dogs

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SYNOPSIS
Bone morphogenetic protein-2 (BMP-2) may play a major role in osteoinduction and osteoclast differentiation. The influence of BMP-2 conditioning in graded doses to root dentin surface on periodontal wound healing was examined.

Twenty-four periodontal defects were surgically created on the buccal roots of four beagle dogs. The denuded root dentin surfaces were demineralized with 24% EDTA, 0.1 and 1.0 µg/µL BMP-2 solution (15 µL) was applied, and the groups were labeled as low-dose BMP (BL) and high-dose BMP (BH), respectively. In the control roots, phosphate-buffered saline was applied to the root surface. Specimens were histologically analyzed 16 weeks after surgery.

Periodontal wound healing was stimulated by conditioning with BMP-2. Formation of alveolar bone and cementum-like tissue was observed; however, in BH group, ankylosis and root resorption were accelerated in the defect area, and there was little evidence of periodontal ligament formation between alveolar bone and root surface.

***Key words**: bone morphogenetic protein-2, root dentin surface conditioning, periodontal wound healing, dog*

INTRODUCTION

In periodontal therapy, it is generally considered that functional periodontal tissue can be regenerated by accelerating periodontal ligament cell and osteogenic cell repopulation. Various growth factors have been used for modification of the periodontal regenerative therapy. Specifically, bone morphogenetic protein-2 (BMP-2) has the ability to transform pluripotent stem cells into osteoprogenitor cells [1] and induce osteogenic effects and bone induction [2, 3].

Previous studies have reported that BMP-2 could be retained by an EDTA-demineralized dentin surface, and the osteogenic activity of human periodontal ligament cells on BMP-applied dentin is stimulated [4].

Miyaji *et al.* designed a study in which surface of dentin block was treated with BMP-2 and implanted in rat connective tissue where little osteogenic tissue was formed subsequently. They observed formation of cementum-like tissue on most of the BMP-loaded dentin block surfaces [5]. Therefore, we hypothesized that periodontal wound healing associated with bone and cementum formation might be enhanced by BMP-2 loaded on root dentin surface in the periodontal tissue.

BMP-2 may play a critical role by interfering with the coupling pathways for osteoblasts and osteoclasts. Recent studies using dentin pit resorption assay have demonstrated that BMP-2 significantly stimulated osteoclast differentiation and osteoclastic resorbing activity [6, 7]. An *in vivo* study in rats demonstrated that dentin resorption was induced by BMP-2 loading [8]. The relationship between BMP-2 loading dose and osteoclastic activity was also analyzed histomorphometrically; BMP-2 caused a dose-related increase in dentin resorption, however, the osteogenic effect of BMP-2 in high doses was dose-dependent in a reverse mode. Therefore, root resorption is conceivably modulated by variations in the dosage of BMP-2 in periodontal BMP-2 therapy. Root surface treatment with relatively high doses of BMP-2 may promote osteoclastic activity in addition to inducing periodontal tissue formation.

When combined with a biological carrier system, BMP-2 facilitated alveolar bone augmentation in periodontal defects [9-14]. However, for BMP conditioning method, information on the periodontal wound healing have not yet been provided. The aim of the present *in vivo* study was to examine the effects of root dentin surface conditioning with BMP-2 on periodontal wound healing in periodontal defects in beagle dogs.

MATERIALS AND METHODS

Animals

Four healthy female beagle dogs, 10-12 months old, were used in this experiment. The experimental protocol (No. 5098) followed the Guidelines for the Care and Use of Laboratory Animals of the Graduate School of Medicine, Hokkaido University. Surgical procedures were performed under general anesthesia with medetomidine hydrochloride (0.1 mL/kg, Domitor, Meiji seika, Tokyo, Japan) and ketamine hydrochloride (0.1 mL/kg, Ketaral 50, Sankyo, Tokyo, Japan), and local anesthesia with lidocaine hydrochloride (2% with 1:80,000 epinephrine, Xylocaine, AstraZeneca, Osaka, Japan).

BMP-2 construct

Recombinant human BMP-2 (98% purity) was donated by Astellas Pharma (Tokyo, Japan). The BMP-2 was diluted with phosphate-buffered saline (PBS; 5 mM, pH 7.2) to produce stock solutions of 0.1 and 1.0 µg/µL.

Surgical procedure

Following reflection of a partial thickness flap on the buccal side of the 2nd and/or 3rd maxillary and mandibular premolars, alveolar bone and periosteum to a depth of 6 mm (measured from the cemento-enamel junction) were removed using a rotating round bur under water irrigation. The root surface facing the defect was planed to remove cementum. Reference notches indicating the cemento-enamel junction and bottom of the defect were prepared on the root surfaces. Thirty-two periodontal dehiscence type defects were thus surgically created (Fig. 1-A, B). Defects in alternate quadrants in dogs were then randomly assigned to each group. Subsequently, the denuded root surface was demineralized with 24% EDTA (pH 7.0) for 3 min, and washed with saline. 15µL of BMP-2 solution (loading dose; 0.1

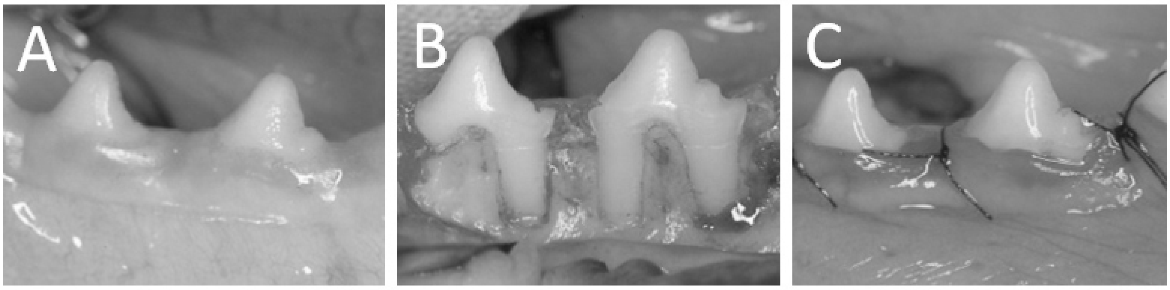

Figure 1
(A) Pre-surgery view of the experimental area. (B) Dehiscence type periodontal defects were created. (C) After BMP application to root dentin surface, the flaps were repositioned and sutured.

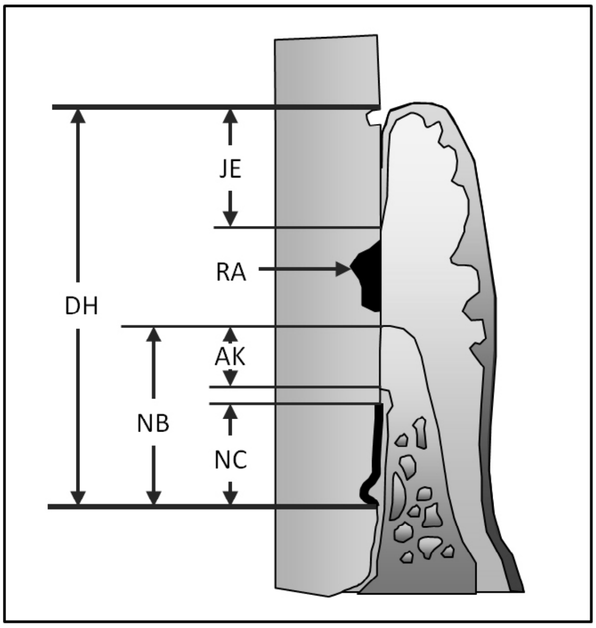

Figure 2
Schematic drawing of histomorphometric analysis of periodontal wound healing. (DH, defect height; NB, new bone; NC, new cementum; JE, junctional epithelium; AK, ankylosis; RA, root resorbed area.)

and 1.0 µg/µL) was applied to the root dentin surface by pipette and acrylic resin sponge. The defect sites receiving BMP-2 at 0.1 and 1.0 µg/µL were labeled as BMP at low-dose (BL) and BMP at high-dose (BH) groups, respectively. As control, PBS alone was applied to the root surface. The flap was repositioned and securely sutured (Surgilon, Tyco Healthcare Japan, Tokyo, Japan, Fig. 1-C). The animals received ampicillin sodium (300 mg/kg, Viccillin, Meiji seika) daily for 3 days and a plaque control regimen with 0.5% chlorhexidine twice weekly, for the entire period of the experiment.

Histological procedure

The animals were euthanized using an overdose of sodium pentobarbital (0.5 mL/kg, Nembutal injection, Abbott Laboratories, IL, USA) following general anesthesia. Specimens were collected from the wound 16 weeks post-surgery. The tissue blocks, including teeth, bone and soft tissue, were fixed in 10% buffered formalin, decalcified in 10% formic-citric acid, and embedded along the buccolingual plane in paraffin wax. Six-micrometer-thick sections were serially prepared and stained with hematoxylin-eosin (HE).

Histomorphometric analysis

Three HE-stained sections were taken; one was approximately from the center of the root, and the other two were 100 µm from either side of the center. The following 6 histomorphometric measurements (Fig. 2) were performed for each stained section using a software package (Scion image, Scion, MD, USA) :

1. Defect height: Distance between the apical notch and the cemento-enamel junction.
2. New bone: Percentage of the length of newly formed alveolar bone in relation to the defect height.

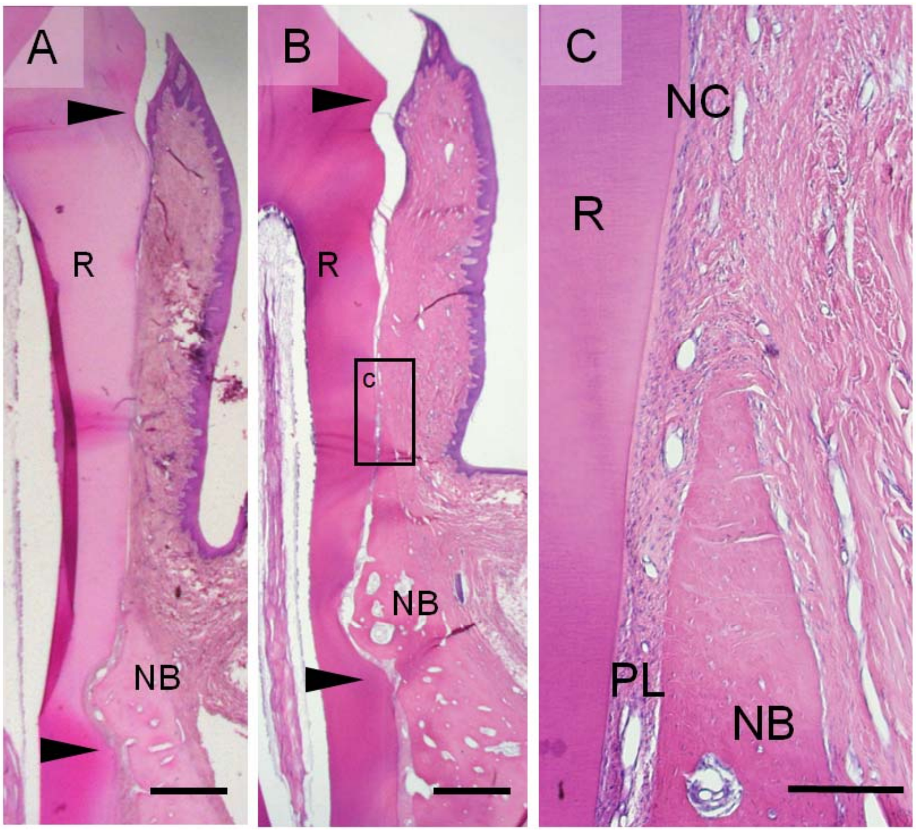

Figure 3
(A) Histological findings in the control. The coronal and apical notches were indicated by arrowheads. Gingival connective tissue was attached to the root surface without bone and cementum formation. New bone was demonstrated only in the apical portion of the defect. (B) Histological findings in the BL group. The coronal and apical notches were indicated by arrowheads. Alveolar bone and cementum formed parallel to the root surface. (C) Higher magnification of the framed area (c) in (B). Periodontal ligament tissue was observed between alveolar bone and cementum. (R, root; NB, new bone; NC, new cementum; PL, periodontal ligament; HE staining; scale bars: A = 1 mm; B = 1 mm; C = 200 µm.)

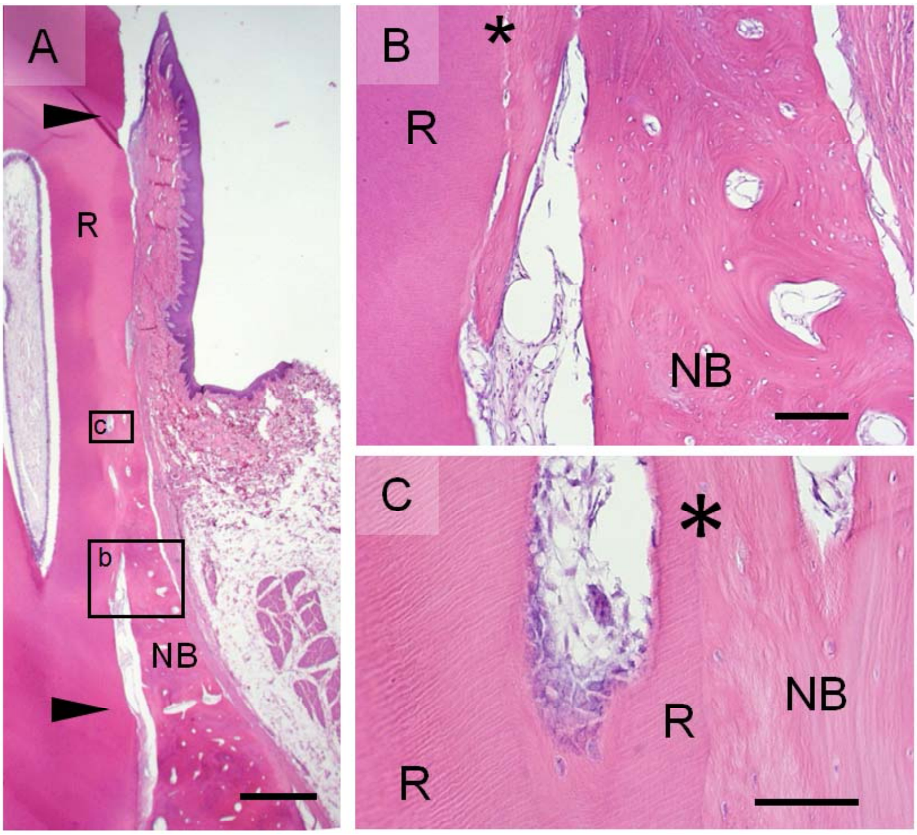

Figure4
Histological findings in the BH group. (A) Alveolar bone was formed in most of the defect area. The coronal and apical notches were indicated by arrowheads. (B) Higher magnification of the framed area (b) in (A). Ankylosis (star) were accelerated in the defect area. (C) Higher magnification of the framed area (c) in (A). Osteoclastic cells associated with ankylosis and extensive root resorption were observed frequently. (R, root; NB, new bone; HE staining; scale bars: A = 1 mm; B = 200 µm; C = 100 µm.)

3. New cementum: Percentage of the length of newly formed cementum and cementum-like tissue on the root surface in relation to the defect height.
4. Junctional epithelium: Percentage of the length of junctional epithelial downgrowth on the root surface in relation to the defect height.
5. Ankylosis: Percentage of the length of ankylosis on the root surface in relation to the defect height.
6. Root resorbed area: The gross area of resorbed root dentin facing the defect area in relation to the defect height.

Statistical analysis

The means and standard deviations of each parameter were calculated for each group. Differences among the groups were analyzed using the Games-Howell test. P-values $<$ 0.05 were considered statistically significant. All statistical procedures were performed using a software package (SPSS 11.0, SPSS Japan, Tokyo, Japan).

RESULTS

Histological observations

Due to pulp tissue exposure during the surgical procedure and tissue processing error, two specimens were eliminated from this study. Otherwise, postoperative healing was uneventful in all dogs.

In the control (Fig. 3-A), newly formed alveolar bone, cementum and periodontal ligament continuing from original periodontal tissue were observed only in the apical portion of the defect. There was no ankylosis, but mild root resorption was noted and gingival connective tissue was seen attached to the root surface in most areas. Downgrowth of the junctional epithelium was frequently demonstrated in the coronal portion of the root surface.

In the BL group (Fig. 3-B), alveolar bone and cementum formation occurred parallel to the root surface. Newly formed bone comprised osteocyte and trabecula lined by osteoblastic cells. Periodontal ligament tissue was reestablished with functionally oriented fibers between alveolar bone and cementum (Fig. 3-C). Mild root resorption was frequently noted; however, osteoclastic cells were rarely observed in the resorbed area. Ankylosis could not be detected in any of the specimens.

In the BH group (Fig. 4-A), although alveolar bone and cementum-like cellular tissue formation had occurred in most of the periodontal defect area, ankylosis with root dentin resorption was noted in contrast to control and group BL. All roots exhibited evidence of ankylosis. A periodontal ligament space between the alveolar bone and root surface / cementum-like tissue was evident, however, cells and extracellular matrix were poorly distributed at the interface (Fig. 4-B). Osteoclastic cells were predominant in the ankylotic sites (Fig. 4-C). Epithelial downgrowth was suppressed at the coronal portion of the root surface.

Histomorphometric analysis

Significantly more new bone formation was shown in groups BL and BH than in control. Cementum formation in group BH was significantly greater than those in the control. Downgrowth of the junctional epithelium in the BH group was significantly less than that in control. Evidence of ankylosis was not observed in control and BL group; however, ankylosis in group BH significantly extended to approximately 20% of the root surface. The root resorbed area in group BH was measured maximally and found to be significantly greater than that in control (Table 1).

Table 1 Histomorphometric analysis at 16 weeks after BMP-2 application (mean ± SD).

	Control (n=8)	Group BL (n=7)	Group BH (n = 7)
New bone (%)	37.7 ± 12.6	52.6 ± 10.9*	65.4 ± 16.6*
New cementum (%)	50.9 ± 16.4	66.0 ± 18.2	70.1 ± 14.2*
Junctional epithelium (%)	22.3 ± 8.4	11.8 ± 11.1	7.9 ± 7.0*
Ankylosis (%)	0.0 ± 0.0	0.0 ± 0.0	26.1 ± 18.5*†
Root resorbed area (mm^2/mm)	0.016± 0.007	0.037 ±0.034	0.071 ± 0.039*

* Statistical difference compared to control ($p < 0.05$).

† Statistical difference compared to group BL ($p < 0.05$).

DISCUSSION

The present study focused on periodontal wound healing following root surface conditioning with BMP-2. Histological findings revealed that BMP-2 conditioning to the root dentin surface stimulated periodontal wound healing. In particular, alveolar bone and cementum induction was consistently accelerated by BMP-2 treatment. It was suggested that cell proliferation, differentiation and tissue formation in the periodontal defect were augmented by BMP-2 released from the root dentin surface. The present examination revealed that ankylosis was induced in BH group. Ordinarily, ankylosis is inhibited by regeneration of periodontal ligament simultaneously. This examination also showed that reestablishment of the periodontal ligament structure was rich in group BL, but poor in group BH. Therefore, we speculate that high dose of BMP-2 suppresses the proliferation of the periodontal ligament cells onto instrumented root surface. Zaman *et al.* reported that BMP-2 could not enhance the proliferative activity of periodontal ligament cell [4]. Periodontal ligament cell repopulation in BMP therapy should be accelerated by additional implantation of regenerative material with several growth factors [15, 16]. On the other hand, Takahashi *et al.* demonstrated that resolution of ankylosis by osteoclastic cells was encountered in a cat study, and explained that occlusal loading could be available with prevention of ankylosis formation [14]. Ankylosis resulting from BMP-2 use is an important point to be verified in the future.

Osteoclastic resorption and deposition of repair cementum would occur at the root surface in the early wound healing of periodontal tissue without BMP-2 use [17, 18]. In this study, root resorbed area in group BH was significantly greater compared to control, and histological findings indicated that osteoclastic activity was promoted with the application of the high dose BMP to the root dentin surface. Recent *in vitro* studies have shown that BMP-2 significantly stimulated osteoclast differentiation and osteoclastic resorbing activity [19], and Miyaji *et al.* elucidated that dentin block resorption by osteoclastic cells was promoted by the application of high doses of BMP-2 to the dentin surface in rat connective tissue [8]. Therefore, the results in beagle dogs suggest that a relatively high dose of BMP-2 may preserve a long-term high level of resorbing activity in osteoclastic cells and significantly induce root resorption.

It has been reported that EDTA treatment could remove the surface smear layer, open the dentin tubules and expose the collagen matrix without inducing morphological change when applied to an instrumented dentin surface [20]. The extracellular matrix is an attractive target for localizing growth factors as therapeutic agents. Platelet-derived growth factor loaded on the root surface demineralized with citric acid enhanced periodontal regeneration [21]. Since BMP-2 has the ability to bind to the collagen matrix, the root surface modified by demineralization may exhibit properties of a carrier of BMP-2. A previous study demonstrated that the ALPase activity of human periodontal cells attached to dentin surfaces treated by both EDTA and BMP-2 was higher than on dentin surfaces treated by EDTA alone [4, 22]. Tenkumo *et al*. prepared dentin blocks, which were treated with EDTA or PBS, and then applied BMP-2 solution. The dentin blocks were later implanted in rat connective tissue. Significant cementum-like tissue formation was facilitated on the EDTA-treated dentin group, while no formation of cementum-like tissue was found in the PBS treatment group [23]. These findings suggest that BMP-2 cannot be held on the root dentin surface without treatment for collagen fibril exposure.

CONCLUSION

Root dentin surface conditioning with BMP-2 could have distinct effect on periodontal wound healing in beagle dogs. It was also demonstrated that the osteogenic and resorptive effects of BMP-2 are dose dependent. Ankylosis and root resorption were facilitated with a relatively high dose of BMP-2.

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