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Periodontal repair in dogs: effect of recombinant human bone morphogenetic protein-12 (rhBMP-12) on regeneration of alveolar bone and periodontal attachment

A pilot study

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Abstract

Objectives: Recombinant human bone morphogenetic protein-2 (rhBMP-2) has been shown to stimulate alveolar bone and cementum formation in periodontal defects but not a functionally oriented periodontal ligament (PDL). Subcutaneous and intramuscular implants of BMP-12 have been shown to induce tendon formation and ligament-like tissue. The objective of this study was to evaluate rhBMP-12 for periodontal regeneration, in particular PDL formation.

Methods: Six young adult Hound Labrador mongrel dogs were used. Routine supraalveolar periodontal defects were created around the mandibular premolar teeth. Three animals received rhBMP-12(0.04 mg/ml) in an absorbable collagen sponge (ACS) carrier vs. rhBMP-12(0.2 mg/mL)/ACS in contralateral defects. Three animals received rhBMP-12(1.0 mg/ml)/ACS vs. rhBMP-2(0.2 mg/ml)/ACS (total implant volume/defect $\sim$1 ml). The animals were euthanized 8 weeks postsurgery and block biopsies were processed for histometric analysis.

Results: Bone regeneration appeared increased in sites receiving rhBMP-2/ACS compared to sites receiving rhBMP-12/ACS. Cementum regeneration was similar comparing sites implanted with rhBMP-2/ACS to sites implanted with rhBMP-12/ACS. In contrast, sites receiving rhBMP-12/ACS exhibited a functionally oriented PDL bridging the gap between newly formed bone and cementum whereas this was a rare observation in sites receiving rhBMP-2/ACS. Ankylosis appeared increased in sites receiving rhBMP-2/ACS compared to those receiving rhBMP-12/ACS.

Conclusions: The outcomes of this study suggest that rhBMP-12 may have significant effects on regeneration of the PDL. Additional preclinical evaluation is needed to confirm these initial observations prior to clinical application.

Key words: cementum; dogs; periodontal ligament; periodontal regeneration; tissue engineering

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Recombinant human bone morphogenetic protein-2 (rhBMP-2) has been shown to stimulate clinically significant regeneration of alveolar bone and cementum in experimental periodontal defects (Ishikawa et al. 1994, Sigurdsson et al. 1995a, b, 1996, Kinoshita et al. 1997, Wikesjö et al. 1999, 2003a–c, Choi et al. 2002, Selvig et al. 2002). Induced bone appears to integrate with the resident bone. Radiographic and histologic evaluations suggest that the newly formed bone exhibits characteristics of the contiguous resident bone. However, rhBMP-2 treatment does not appear to induce a functionally oriented periodontal ligament (PDL) and frequently results in ankylosis (Sigurdsson et al. 1995a, b, 1996, King et al. 1997, 1998a, b, King & Hughes 1999, 2001, Wikesjö et al. 1999, 2003a–c, Talwar et al. 2001, Selvig et al. 2002). Wikesjö et al. (1999) reported ankylosis in animals receiving rhBMP-2 in an absorbable collagen sponge (ACS) carrier without correlation to rhBMP-2 dose. The ankylotic union between the teeth and the newly formed bone was commonly observed in the coronal aspect of the supraalveolar defects evaluated. Cellular cementum, extending from the apical extension of the defect, often merged with the ankylotic bone. Similar observations have been made for rhBMP-2 in other candidate carriers (Sigurdsson et al. 1995a, b, 1996, Wikesjö et al. 2003a) and for osteogenic protein-1 (OP-1/BMP-7) (Ripamonti et al. 1996, Giannobile et al. 1998). In the absence of extensive bone regeneration, commonly in more limited periodontal defects, ankylosis has not been a dominant observation (Ishikawa et al. 1994, Kinoshita et al. 1997, Blumenthal et al. 2002, Choi et al. 2002). In these cases, cementum regeneration with a fibrous attachment may be observed.

BMP-12, a member of the transforming growth factor-β/BMP gene family (Chang et al. 1994, Wolfman et al. 1997), is currently being evaluated for tendon and ligament repair. BMP-12 is the human homologue of mouse growth/differentiation factor-7 (GDF-7) (Hattersley et al. 1998). GDF-5, -6, or -7 have been found to induce connective tissue formation rich in type I collagen fibers resembling neonatal tendon and ligament when implanted in vivo. Analysis of the expression pattern of GDF-5, -6, or -7 suggests that they act as signaling molecules during embryonic tendon, ligament, and joint formation (Wolfman et al. 1997). In the tissue induced by this BMP subgroup, proteins specific to bone (osteocalcin, alkaline phosphatase) are absent but those specific to tendon and ligament are present (Inada et al. 1996, Wolfman et al. 1997, Hattersley et al. 1998). It is believed that the intracellular signaling pathway of BMP-12 is different from that of BMP-2. However, Furuya et al. (1999) reported increased alkaline phosphatase activity in rat osteoblastic osteosarcoma ROS 17/2.8 cells in the presence of BMP-12. Valcourt et al. (1999) reported differences in the expression of BMP-2 or -4 and BMP-12 or -13 in the MC615 chondrocyte cell line. The level of type II collagen mRNA was increased in the presence of BMP-2 or -4 but no effect was seen in the presence of BMP-12 or -13. Expression of the matrix Gla protein gene, a cartilage marker, was decreased in the presence of BMP-2 or -4 but stable in the presence of BMP-12 or -13. Bone Gla protein, a bone phenotype marker, was induced in the BMP-2 or -4 treated cells but not detected in the BMP-12 or -13 treated cells. MC615 chondrocytes also express chondrocytic and osteoblastic markers in the presence of BMP-2 or -4 but not to BMP-12 or -13. This is consistent with previous in vivo studies showing the osteoinductive properties of BMP-2 and -4 (Wozney 1998) and neotendon/ligament induction without bone formation with BMP-12 and -13 (Wolfman et al. 1997).

Subcutaneous and intramuscular implants of BMP-12 (1–100 μg) have been found to induce formation of tendon and ligament tissue in the adult rat (Wolfman et al. 1995, 1997). BMP-12 and rhBMP-2 have been evaluated in the rat tendon-bone attachment model. BMP-12-treated sites induced a new attachment with a distinct fibrocartilaginous zone at the bone–tendon interface. The untreated tendons showed poor healing response and failure to reform a morphologically normal attachment site. In that study, rhBMP-2 led to tendon ossification and narrow fibrocartilaginous interface with a lower failure load (Hattersley et al. 1998). Formation of neotendon/ligament and bone resulted when BMP-12 and rhBMP-2 were implanted together. High doses of BMP-12 and -13 ($>100\,\mu$g) induced ectopic bone formation in vivo and direct injection into adult rat tendons/ligaments resulted in new connective tissue and endochondreal ossification. The objective of this study was to evaluate the effect of rhBMP-12 on regeneration of alveolar bone and cementum, and in particular PDL formation.

Material and Methods

Animals

Six male Hound Labrador mongrel dogs, age 18–24 months, weight approximately 20 kg, exhibiting intact mandibular premolar dentition without crowding or evidence of periodontal disease, obtained from an USDA-approved dealer, were used. Animal selection and management, surgery protocol, and periodontal defect preparation followed routines approved by the Animal Care and Use Committee, Wyeth Research, Cambridge, MA, USA. The animals had access to standard laboratory diet and water until the beginning of the study. Oral prophylaxis was performed within 2 weeks prior to the experimental surgeries.

BMP constructs

Using aseptic routines rhBMP-12 (Wyeth Research), supplied at a concentration of 1.5 mg/ml, was diluted with MFR 00906 buffer (0.5% sucrose, 2.5% glycine, 30 mM L-glutamatic acid, 0.01% polysorbate 80, pH 4.5; Wyeth Research) to produce rhBMP-12 stock dilutions at 0.04, 0.2, and 1.0 mg/ml. Lyophilized rhBMP-2 (Wyeth Research) reconstituted to 4.45 mg/ml liquid concentration was diluted with MFR 00906 buffer to produce a rhBMP-2 stock dilution at 0.2 mg/ml.

For the manufacture of the BMP constructs a $1 \times 2''$ absorbable collagen sponge (Helistat® ACS, Integra Life Sciences, Plainsboro, NJ, USA) was placed onto a sterile field. The sponge was cut into equal halves and one half was discarded. A 0.65-ml aliquot of the rhBMP-12 or rhBMP-2 stock solutions was uniformly dispensed over the entire surface of the $1 \times 1''$ sponge. The rhBMP-12/ACS or rhBMP-2/ACS constructs remained covered for 30 min to allow for incorporation of rhBMP-12 or rhBMP-2. Tissue binding studies have indicated similar binding times for rhBMP-2 and rhBMP-12. The prepared rhBMP- 2/ACS or rhBMP-12/ACS constructs were cut into pieces to fit the supraalveolar periodontal defect.

Surgical procedure

Food was withheld the night preceding surgery. Animals were pre-anesthetized with buprenorphine HCl (0.01–0.03 mg/kg)/acepromazine (0.1 mg/kg)/atropine (0.02–0.04 mg/kg) SQ, sedated with methohexital (4–8 mg/kg to effect), and maintained on gas anesthesia (1–3% isoflurane/O_2 to effect). To maintain hydration, a sterile I.V. catheter was placed and animals received a constant rate infusion of lactated Ringer's solution (10–20 ml/kg/h I.V.) while anesthetized. Prophylactic antibiotics (cefazolin; 22 mg/kg SQ) were administered within 1 h of surgery and redosed postsurgery.

In the maxilla, buccal sulcular incisions were made from the canine to the fourth premolar to reflect buccal mucoperiosteal flaps. The first, second and third premolar teeth were extracted bilaterally, and the fourth premolars were reduced in height and exposed pulpal tissues sealed (Cavit®, ESPE, Seefeld/Oberbayern, Germany) to alleviate potential trauma from the maxillary teeth to the experimental mandibular sites postsurgery. After extractions, the flaps were re-apposed and sutured (GORE-TEX™ Suture CV5, W.L. Gore & Associates Inc., Flagstaff, AZ, USA) ensuring primary wound closure.

Supraalveolar, critical size, periodontal defects were created around the third and fourth mandibular premolar teeth in the right and left jaw quadrants following buccal and lingual mucoperiosteal flap elevation (Wikesjö et al. 1994). Briefly, alveolar bone was removed around the circumference of the teeth with chisels and water-cooled rotating burs. The first and second premolars were extracted bilaterally, and the first molars were amputated at the level of the reduced alveolar crest. The root surfaces of the third and fourth premolar teeth were instrumented with curettes, chisels, and water-cooled rotating diamonds to remove the cementum. The crowns of the teeth were reduced to approximately 2 mm coronal to the cemento-enamel junction (CEJ) and the cut surfaces smoothed. Exposed pulpal tissues were sealed (Cavit®). Clinical defect height from the CEJ to the reduced alveolar crest was set to 6 mm as measured with a periodontal probe (Fig. 1).

Wound management

Three animals received rhBMP-12(0.04 mg/ml)/ACS versus rhBMP-12 (0.2 mg/ml)/ACS in contralateral supraalveolar periodontal defects and three animals received rhBMP-12(1.0 mg/ml)/ACS versus rhBMP-2(0.2 mg/ml)/ACS (total implant volume/defect $\sim$ 1 ml). The rhBMP-12/ACS or rhBMP-2/ACS construct was fitted into the furcation and interproximal areas and layered to cover the buccal and lingual aspects of the premolar teeth (Fig. 1). Following placement of rhBMP-12/ACS or rhBMP-2/ACS, the periostea were fenestrated at the base of the flaps to allow tension-free flap apposition. The flaps were advanced and the flap margins adapted 3–4 mm coronal to the teeth and sutured (GORE-TEX™).

Postsurgery care

The animals were fed a canned soft dog food diet. Buprenorphine HCl (0.015 mg/kg I.M. bid for 48 h) was administered for pain control. A broad-spectrum antibiotic (enrofloxacin, 2.5 mg/kg, I.M., twice daily for 14 days) was used for infection control. Plaque control was maintained by once daily topical application of chlorhexidine (Chlorhexidine Gluconate 20%, Xttrium Laboratories, Inc., Chicago, IL, USA; 40 ml of a 2% solution) until gingival suture removal, thereafter, once daily (Monday through Friday) until the completion of study. Gingival sutures were removed at approximately 8 days postsurgery.

Clinical recordings

Observations of experimental sites with regards to gingival health, maintenance of suture line closure, edema, and evidence of tissue necrosis or infection were made daily until suture removal, and at least twice weekly thereafter. Radiographs were obtained immediately postsurgery, and at 4 and 8 weeks postsurgery.

Histological processing

At 8 weeks postsurgery, the animals were pre-anesthetized with buprenorphine HCl (0.01–0.03 mg/kg)/acepromazine (0.1 mg/kg)/atropine (0.02–0.04 mg/kg), anesthetized with pentobarbital (30 mg/kg IV bolus), and euthanized with Euthanasia-5 solution I.V. (1 ml/10 kg; Henry Schein, Port Washington, NY, USA). Following euthanasia, block sections including teeth, bone and soft tissues were collected and radiographed to estimate bone regeneration. The specimens were rinsed in sterile saline, sectioned, and fixed in 10% neutral buffered formalin for 8–10 weeks.

The tissue blocks were trimmed, washed, and subsequently decalcified with EDTA (Luna 1992). The specimens were then washed, dehydrated with gradients of alcohol, and cleared in xylene using an automatic tissue processor (Tissue-Tek; Sakura, Torrance, CA, USA). Specimens were infiltrated and embedded in methylmethacrylate allowed to polymerize for 3–5 days at room temperature. Using a Reichert Jung Polycut (Leica, Deerfield, IL, USA), 5-μm sections were taken 100 μm apart through the root canal area and stained with a modified Goldner's trichrome stain.

Analysis

The most central stained section of each root for the third and fourth premolar teeth were identified by the size of the root canal. This section and the immediate stained step serial section on either side were subjected to histometric analysis. Thus, three subsequent step serial sections, representing 0.2 mm of the mid-portion of the mesial and the distal root for each premolar tooth, were used for analysis. One masked experi-

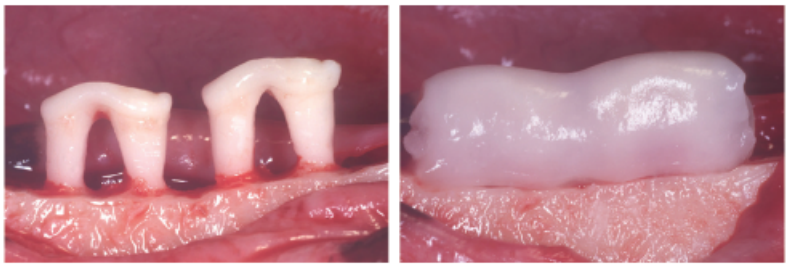

Fig. 1. Critical size, supraalveolar periodontal defect before and after application of rhBMP-12/ACS. The surgical reduction of the native periodontal attachment and alveolar bone approximates 6 mm.

enced examiner using an image analysis software (Image-Pro Plus™, Media Cybernetic, Silver Springs, MD, USA) with a custom program for the supra-alveolar periodontal defect model performed the histometric analysis. The following measurements were recorded for the buccal and lingual tooth surfaces of each section:

- Defect height: distance between apical extension of root planing and CEJ.
- Cementum regeneration (continuous): distance between apical extension of root planing and coronal extension of a continuous layer of new cementum or cementum-like deposit on the planed root.
- Cementum regeneration (total): distance between apical extension of root planing and coronal extension of new cementum or cementum-like deposit on the planed root.
- Bone regeneration (height): distance between apical extension of root planing and coronal extension of new alveolar bone along the planed root.
- Bone regeneration (area): area represented by new alveolar bone along the planed root.
- Bone regeneration (density): ratio of regenerated bone/marrow spaces.
- Root resorption: combined linear heights of distinct resorption lacunae on the planed root.
- Ankylosis: combined linear heights of ankylotic union between new alveolar bone and the planed root.

Summary statistics (means ± SD) based on animal means for the experimental conditions were calculated using the selected step serial sections.

Presence of a periodontal attachment with functionally oriented fibers inserting into the newly formed cementum was examined twice, independently, by two masked experienced examiners. Briefly, the specimens were viewed under polarized light at × 10 and × 20. The region of interest was the space between the newly formed bone and dentin or new cementum. Each specimen was scored as outlined in Table 1. The final score for each root was the average of the scores from the buccal and lingual aspect of the root. Summary statistics (means ± SD) based on animal means for the experimental conditions were calculated using the duplicate registrations for each root.

Table 1. Outline of periodontal ligament scores

Score	Fiber density	Fiber attachment
0	none	none
1	very low	attached to bone only
2	low	attached to bone only
3	low	attached to dentin or new cementum
4	mid	attached to dentin or new cementum
5	high/same as native PDL	attached to dentin or new cementum

Statistical testing of differences between treatment conditions was not performed due to the small sample size.

Results

Clinical and radiographic observations

All animals, irrespective of treatment or dose, exhibited defect exposure generally limited to the cut top surface of the teeth, accompanied by some redness and swelling. Gingival exposures were observed as early as day 4 and as late as day 28 postsurgery. One site healed without exposure. This site, implanted with rhBMP-2/ACS, showed decreasing swelling and was hard on palpation by day 10 postsurgery. To reduce chances of infection, systemic antibiotic and chlorhexidine regimens were extended until the end of study.

Radiographic evidence of bone formation in sites implanted with rhBMP-12/ACS is shown in Fig. 2. Bone formation ranged from 0–20% of the defect height at sites receiving rhBMP-12 at 0.04 mg/ml, 0–40% of the defect height at sites receiving rhBMP-12 at 0.2 mg/ml, and approximated 10–30% of the defect height at sites implanted with rhBMP-12 at 1.0 mg/ml. One defect showed evidence of root resorption in the furcation area. Two sites exhibited evidence of root resorption at the cervical aspect of the teeth. Sites receiving rhBMP-2/ACS exhibited comparatively enhanced bone formation (Fig. 2). Two sites showed bone formation extending above the CEJ. One of these sites exhibited bone voids indicative of seroma formation over the cut coronal surface of the teeth (Fig. 2). The third site receiving rhBMP-2/ACS showed evidence of bone fill approximating 50% of the defect height and evidence of significant root resorption at the cervical aspect of the teeth.

Histological evaluation

Representative photomicrographs for defect sites receiving rhBMP12/ACS and rhBMP-2/ACS are shown in Figs. 3 and 4. Sites implanted with rhBMP-12/ACS or rhBMP-2/ACS exhibited new bone formation of varying extents assuming characteristics, trabeculation and cortex formation of the contiguous resident bone. In general, bone formation at sites receiving rhBMP-2/ACS was more extensive than that at sites receiving rhBMP-12/ACS. There were no remarkable differences in bone formation between sites receiving rhBMP-12/ACS at the various rhBMP-12 concentrations. The newly formed bone usually adapted a ''physiologic form'' along the root surface except for one site implanted with rhBMP-2/ACS where bone formation was considerably more extensive.

A PDL space was observed between the newly formed bone and the previously denuded root surface for all experimental conditions. Commonly the PDL space was terminated by ankylosis in the presence of extensive bone formation. This observation was shared for sites implanted with rhBMP-2/ACS and rhBMP-12/ACS. A new cellular cementum extending from the apical aspect of the defect was observed for all defect sites without remarkable differences between treatments. In contrast, the fibrovascular tissue within the PDL space in sites receiving rhBMP-12/ACS included functionally oriented collagen fibers apparently extending from the newly formed cementum to the newly formed bone. Sites receiving rhBMP-2/ACS exhibited collagen fibers of varying orientation, commonly aligned along the long-axis of the teeth.

Several defect sites, irrespective of treatment protocol, exhibited exposures of the surgically submerged teeth. Nevertheless, in 8 of the 11 exposed sites the junctional epithelium arrested at the CEJ. In three sites, the junctional epithelium arrested within the coronal aspect of the defect. Most of the defects (11/12) exhibited cervical root resorption. Root resorption of surface erosion character, commonly observed following any regenerative protocol in this

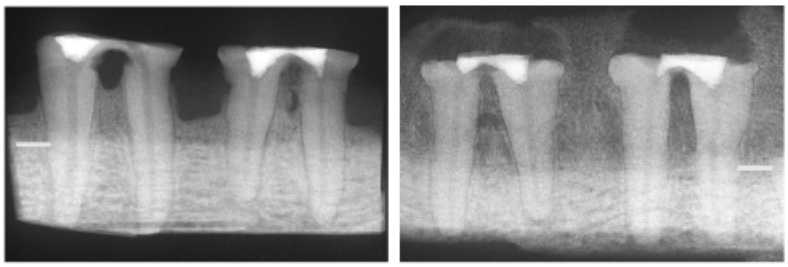

Fig. 2. Healing in contralateral supraalveolar periodontal defects receiving rhBMP-12 (1.0 mg/mL)/ACS (left) or rhBMP-2 (0.2 mg/mL)/ACS (right) at week 8 postsurgery. Note the comparatively robust bone formation in sites receiving rhBMP-2 (0.2 mg/mL)/ACS. The green line indicates the level of the surgical reduction of the native periodontal attachment and alveolar bone.

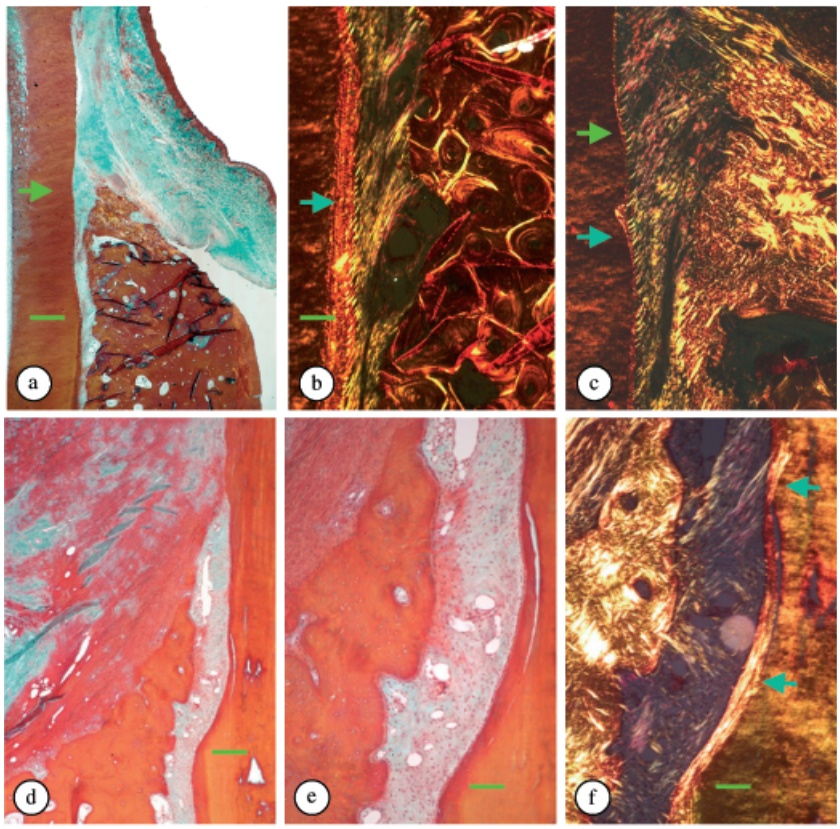

Fig. 3. Photomicrographs of contralateral defect sites receiving rhBMP-12(0.04 mg/ml)/ACS (a–c) and rhBMP-12(0.2 mg/ml)/ACS (d–f). Note new cementum formation including a functionally oriented periodontal ligament. The green line indicates the level of the surgical reduction of the native periodontal attachment and alveolar bone. The green arrow indicates the coronal extension of the newly formed bone in (a) and (c). Blue arrows exemplify areas with a functionally oriented PDL. Original magnifications: × 2.5: a, × 4: d, × 8: b/c, and × 10: e/f; modified Goldner's trichrome stain and polarized light.

animal model, was observed in all defect sites.

Histometric evaluation

The results of the histometric evaluation are shown in Table 2. Bone regeneration averaged 52%, 56%, and 58% of the defect height for sites receiving rhBMP-12/ACS (rhBMP-12 at 0.04, 0.2, and 1.0 mg/ml, respectively). The corresponding value for sites receiving rhBMP-2/ACS approximated 71%. Bone regeneration area was similar among sites receiving rhBMP-12/ACS ranging form 2.1 ± 1.1 to 3.2 ± 2.1 mm^2. Bone regeneration area was numerically higher (5.0 ± 5.0 mm^2), however considerably variable, among sites receiving rhBMP-2/ACS. There were minimal differences in bone density among sites receiving rhBMP-12/ACS or rhBMP-2/ACS; mineralized bone matrix approximated 50% of the new bone area irrespective of treatment. There were also limited differences between treatments relative to cementum formation. Continuous cementum regeneration averaged 1.4 ± 0.8 mm (24% of the defect height) for sites receiving rhBMP-12 (1.0 mg/ml)/ACS. The corresponding values for sites receiving rhBMP-12 (0.04 mg/ml)/ACS, rhBMP-12 (0.2 mg/ml)/ACS or rhBMP-2/ACS were 2.2 ± 1.0 (37%), 2.4 ± 1.3 (41%), and 2.5 ± 1.4 mm (43%), respectively. There were limited differences between treatments relative to total new cementum formation. Similarly, root resorption appeared limited and similar among the treatments. However, ankylosis appeared comparatively increased in sites receiving rhBMP-12 (1.0 mg/ml)/ACS and rhBMP-2/ACS.

The results of the evaluation of the PDL fibers are shown in Table 3. Sites receiving rhBMP-12/ACS exhibited a functionally oriented PDL of relatively high density inserting into newly formed cementum. In contrast, sites receiving rhBMP-2/ACS exhibited collagen fibers of relatively low density oriented in a parallel fashion along the newly formed cementum, inserting into newly formed bone only. There were no remarkable differences between the rhBMP-12 concentrations with the exception that sites receiving rhBMP-12 (0.04 mg/ml)/ACS showed a more consistent reaction than sites receiving rhBMP-12 at higher concentrations.

Discussion

The objective of this study was to evaluate the effect of rhBMP-12 on regeneration of alveolar bone and cementum, and in particular PDL formation. Routine supraalveolar periodontal defects were created around the mandibular premolar teeth in 6 Hound Labrador mongrels. Three animals received rhBMP-12 (0.04 mg/ml)/ACS versus rhBMP-12 (0.2 mg/ml)/ACS, and three animals received rhBMP-12 (1.0 mg/ml)/ACS versus rhBMP-2 (0.2 mg/ml)/ACS in contralateral defects. The animals were euthanized following an 8-week healing interval and block biopsies of the defect sites were processed for histologic and histo-

metric analysis. Bone regeneration appeared increased in sites receiving rhBMP-2/ACS compared to sites implanted with rhBMP-12/ACS. Cementum regeneration was generally similar amongst sites implanted with rhBMP-12/ACS and rhBMP-2/ACS. However, defect sites receiving rhBMP-12/ACS exhibited a functionally oriented PDL bridging the gap between newly formed cementum and alveolar bone whereas this was a rare observation in sites receiving rhBMP-2/ACS.

This study utilized a canine model system including 6-mm, critical size, supraalveolar periodontal defects. The supraalveolar periodontal defect model can be considered a ''litmus test'' for candidate protocols in the evaluation of their potential for regeneration of alveolar bone, cementum, and periodontal attachment (Wikesjö & Selvig 1999). The defect dimensions provide for clinically relevant regeneration of alveolar bone and cementum. The defect morphology allows for an unbiased strategy of analysis as detailed herein and elsewhere (Wikesjö et al. 1994). Alveolar bone and cementum regeneration has been shown not to exceed 15% of the defect height over an 8-week healing interval in sham-surgery controls. Another characteristic of healing in sham-surgery controls is the lack of regeneration of a periodontal attachment with functionally oriented fibers inserting into the new cementum, as seen in the native periodontal attachment. In contrast, the new attachment is predominately characterized by fibrovascular tissue including fibers oriented along the long-axis of the root surface with or without apparent new cementum formation. Substantial regeneration in this model system warrants clinical pursuit of the protocol evaluated, while limited regeneration would be less deserving. Our extensive experience with this model system prompted us to not include a sham-surgery or buffer/ACS control in this initial study.

Previous studies have used the supraalveolar periodontal defect model to assess the effect of various root conditioning protocols, bone derivatives and bone substitutes, growth, differentiation and extracellular matrix factors, devices for guided tissue regeneration (GTR), as stand-alone protocols or in combinations (Wikesjö et al. 1988, 1990, 1991a, b, 1992, 1998, 1999, 2003a–e, Wikesjö & Nilvéus 1990, Haney et al. 1993, Sigurdsson et al. 1994, 1995a, b, 1996, Kim et al. 1998, Trombelli et al. 1999, Tatakis et al. 2000, Selvig et al. 2002). Only the application of GTR as a stand-alone protocol (Haney et al. 1993, Sigurdsson et al. 1994, 1995b, Wikesjö et al. 2003a, b, d) and rhBMP-2 employing various carrier systems (Sigurdsson et al. 1995a, b, 1996, Wikesjö et al. 1999, 2003a–c,

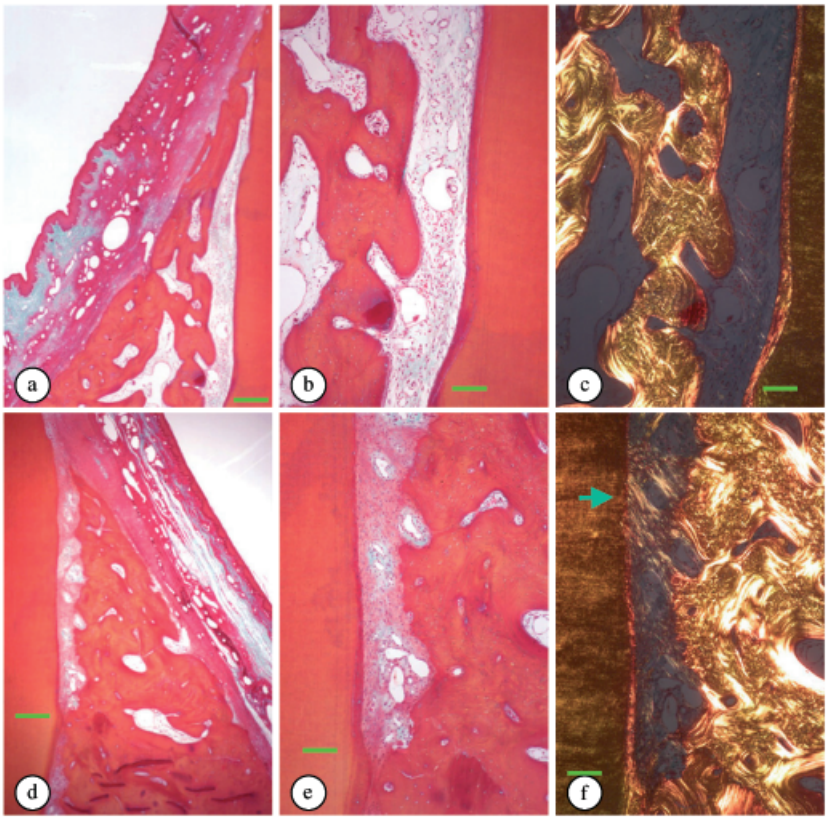

Fig. 4. Photomicrographs of contralateral defect sites receiving rhBMP-2 (0.2 mg/ml)/ACS (a–c) and rhBMP-12 (1.0 mg/ml)/ACS (d–f). Note new cementum formation with an indistinct, limitly appreciable periodontal ligament (PDL) in the site receiving rhBMP-2 (0.2 mg/mL)/ACS. The site receiving rhBMP-12 (1.0 mg/ml)/ACS exhibits new cementum formation with a functionally oriented PDL. The green line indicates the level of the surgical reduction of the native periodontal attachment and alveolar bone. The blue arrow exemplifies areas with a functionally oriented PDL. Original magnifications: × 4: a/d; × 10: b/c/e/f; modified Goldner's trichrome stain and polarized light.

Table 2. Histometric observations for sites receiving rhBMP-12/ACS (rhBMP-12 at 0.04, 0.2, and 1.0 mg/ml) or rhBMP-2/ACS (rhBMP-2 at 0.2 mg/ml)

	Defect height	Bone height	Bone area	Bone density	Cementum continuous	Cementum total	Root resorption	Ankylosis
rhBMP-12(0.04)/ACS	6.0 ± 0.7	3.1 ± 1.9	3.2 ± 2.1	56.7 ± 14.3	2.2 ± 1.0	2.6 ± 1.3	1.0 ± 0.8	0.04 ± 0.2
rhBMP-12(0.2)/ACS	5.9 ± 0.8	3.3 ± 2.2	2.9 ± 2.1	49.6 ± 22.8	2.4 ± 1.3	2.7 ± 1.4	0.9 ± 0.9	0.2 ± 0.4
rhBMP-12(1.0)/ACS	5.9 ± 0.5	3.4 ± 1.3	2.1 ± 1.1	50.9 ± 14.2	1.4 ± 0.8	2.3 ± 0.9	1.2 ± 0.7	0.4 ± 0.6
rhBMP-2(0.2)/ACS	5.8 ± 0.4	4.1 ± 1.6	5.0 ± 5.0	43.5 ± 10.1	2.5 ± 1.4	3.0 ± 1.2	1.4 ± 1.3	0.6 ± 0.7

Group means ± SD (mm); bone area (mm^2), bone density (%).
rhBMP, recombinant human bone morphogenetic protein.

Table 3. Periodontal ligament scores for sites receiving rhBMP-12/ACS (rhBMP-12 at 0.04, 0.2, and 1.0 mg/ml) or rhBMP-2/ACS (rhBMP-2 at 0.2 mg/ml)

Animal	rhBMP-12(0.04)	rhBMP-12(0.2)	rhBMP-12(1.0)	rhBMP-2(0.2)
1	4.8 ± 0.2	4.5 ± 0.2	–	–
2	4.2 ± 0.6	2.9 ± 0.3	–	–
3	4.7 ± 0.4	4.2 ± 0.6	–	–
4	–	–	1.9 ± 0.5	1.4 ± 0.3
5	–	–	3.3 ± 0.8	2.4 ± 0.3
6	–	–	4.0 ± 0.5	1.6 ± 0.3
Group	4.6 ± 0.3	3.9 ± 0.9	3.1 ± 1.1	1.8 ± 0.5

Animal and group means (± SD) from duplicate examinations by two independent examiners. rhBMP, recombinant human bone morphogenetic protein.

Selvig et al. 2002) have been shown to support clinically meaningful regeneration of alveolar bone and cementum. The application of occlusive or novel macro-porous membranes for GTR resulted in clinically relevant regeneration of alveolar bone adopting a ''physiologic form'' along the root surface, formation of a cellular cementum and a functionally oriented PDL with cementum inserting collagen fibers (Sigurdsson et al. 1994, 1995b, Wikesjö et al. 2003a, b, d). Surgical implantation of rhBMP-2 resulted in substantial regeneration of alveolar bone, usually encompassing the entire 5–6-mm supraalveolar defect, and regeneration of cellular cementum although without a functionally oriented PDL (Sigurdsson et al. 1995a, b, 1996, Wikesjö et al. 1999, 2003a–c, Selvig et al. 2002). Following an 8-week healing interval, fibrovascular tissue was observed including collagen fibers that were essentially oriented parallel to the newly formed cementum. With observations extending up to 24 weeks postsurgery, the regenerated cementum was observed interfacing fatty marrow (Wikesjö et al. 2003a). Ankylosis was regularly observed located in the coronal third of the supraalveolar defect. In the present study, the observations at sites implanted with rhBMP-2/ACS are consistent with these observations from previous studies evaluating rhBMP-2 using a variety of carrier technologies.

Surgical implantation of rhBMP-12/ACS followed the pattern of bone and cementum formation observed following implantation of rhBMP-2/ACS; however, bone formation appeared less extensive. Quantitative comparisons to previous studies utilizing the supraalveolar periodontal defect model system with an 8-week interval may not necessarily be meaningful since this study included exposed teeth, whereas previous studies have not included exposed teeth in the analysis. However, the exposures were commonly limited to the most cervical aspect of the defects; the gingival epithelium arrested at or within a short distance from the CEJ. Thus, the healing events produced were sufficient to satisfy the specific objective of this study that is to evaluate the formation of a new PDL. Sites receiving rhBMP-2/ACS exhibited connective tissue fibers of low density mainly attached to the newly formed bone. Sites receiving rhBMP-12/ACS exhibited a new fibrous attachment of comparatively high density attached to newly formed bone, dentin, or new cementum. The observations at sites receiving rhBMP-2/ACS are consistent with that of previous studies evaluating rhBMP-2 technologies in this model system (Sigurdsson et al. 1995a, b, 1996, Wikesjö et al. 1999, 2003a–c, Selvig et al. 2002). The observations of a fibrous attachment inserting into newly formed cementum on the previously denuded root surface are novel and consistent with tissue reactions to this BMP technology in other model systems (Wolfman et al. 1995, 1997). It must be noted however that previous studies have reported formation of a functional periodontal attachment following application of rhBMP-2 or other BMP technologies for periodontal regeneration (Ishikawa et al. 1994, Ripamonti et al. 1994, Kinoshita et al. 1997, Giannobile et al. 1998, Kuboki et al. 1998, Blumenthal et al. 2002, Choi et al. 2002). Such observations may likely be explained by the use of indiscriminant model systems, however, it cannot be ruled out that genuine differences in biologic potential may exist among technologies.

Ankylosis appeared less appreciable particularly in defects receiving rhBMP-12/ACS at 0.04 and 0.2 mg/ml compared to defects receiving rhBMP-12 (1.0 mg/ml)/ACS or rhBMP-2/ACS in this study. However, the study protocol only allowed direct comparisons between rhBMP-12(1.0 mg/ml)/ACS and rhBMP-2/ACS in the same animals, hence this perceived difference may be explained by differences between animals rather than experimental protocol. Nevertheless, root resorption and ankylosis in the cervical third of the defects, a frequent observation in this demanding defect model following surgical implantation of rhBMP-2 (Sigurdsson et al. 1995a, b, 1996 Wikesjö et al. 1999, 2003a–c, Selvig et al. 2002), were commonly observed. Root resorption and ankylosis are rarely encountered in the apical aspect of the supraalveolar periodontal defect and thus do not appear to be a healing aberration in more limited periodontal defects (Kinoshita et al. 1997, Choi et al. 2002). Ankylosis has also not been found to be a healing aberration following surgical implantation of BMPs in the absence of extensive bone regeneration (Ripamonti et al. 1994, Kinoshita et al. 1997, Choi et al. 2002).

Conclusions

The outcomes of this pilot study suggests that rhBMP-12 may have significant effects on the regeneration of the PDL. Additional preclinical evaluation is needed to confirm these initial observations prior to clinical testing.

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