Radiographic Evaluation of Pulpal and Periapical Response of Dogs' Teeth After Pulpotomy and Use of Recombinant Human Bone Morphogenetic Protein-7 as a Capping Agent

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ABSTRACT

Purpose: The purpose of this study was to evaluate radiographically the pulpal and periapical response of dogs' teeth after pulpotomy and the use of recombinant human bone morphogenetic protein-7 (rHuBMP-7).

Methods: Pulpotomies were performed in 60 teeth of 6 dogs, and the remaining radicular pulp tissue was capped with the following materials: (a) groups 1 and 5—recombinant human bone morphogenetic protein-7 associated with recombinant human-like collagen; (b) groups 2 and 6—recombinant human-like collagen; (c) groups 3 and 7—calcium hydroxide; and (d) groups 4 and 8—zinc oxide and eugenol cement. After 7 days (groups 1-4) and 70 days (groups 5-8), standardized periapical radiographs were taken and the integrity of the lamina dura, presence of areas of periapical bone rarefaction, internal/external root resorption, and dentin bridge formation were evaluated. The results were analyzed statistically by Fisher's exact test and Bonferroni correction. The radiolucent areas suggestive of periapical lesions associated with the roots were measured in mm2, and the results were compared by Kruskal-Wallis test.

Results: In the 7-day period, all specimens in groups 1 to 4 presented intact lamina dura and absence of periapical bone rarefaction, internal/external root resorption or dentin bridge formation. In the 70-day period, no specimen in groups 5, 6, and 8 presented dentin bridge formation. Periapical bone rarefaction areas were observed to be associated with 100%, 60%, and 40% of the roots in group 6, 8, and 5, respectively. The largest lesions were found in group 6, followed by groups 5 and 8 ($P<.05$). In group 7, there was dentin bridge formation in 60% of the cases and intact lamina dura and no periapical bone rarefaction in all specimens.

Conclusion: The use of rHuBMP-7/rHuCollagen as a capping material after pulpotomy did not induce mineralized tissue deposition, leading to the formation of radiographically visible periapical lesions. (J Dent Child 2008;75:14-9) Received October 27, 2006 | Last Revision January 25, 2007 | Revision Accepted May 14, 2007.

KEYWORDS: PULPOTOMY, RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN, RECOMBINANT HUMAN COLLAGEN, CALCIUM HYDROXIDE, ZINC OXIDE EUGENOL CEMENT

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Bone morphogenetic proteins (**BMPs**) comprise the largest subfamily of transforming growth factor-β (TGF-β) and belong to a group of noncollagen proteins. The BMPs are widely distributed in mineralized and nonmineralized tissues and have an important role during embryogenesis.[1-3] They are responsible for several biological activities involving tissue morphogenesis, regeneration, healing, and cell differentiation processes.[3] When applied

directly to the pulp tissue, the BMPs dissolve in the tissue fluids and stimulate mitosis of mesenchymal cells and cell differentiation.[4] BMP target-cells in pulp tissue are undifferentiated mesenchymal cells.[3,5,6] These cells present BMP-specific surface receptors to which BMPs bind and ini-tiate a cascade of cellular and biological events that culminate in cell differentiation and production of reparative dentin.[1,2,5,6]

The histopathological response of pulpal and periapical tissues of dogs' teeth after pulpotomy and the use of recombinant human bone morphogenetic protein-7 as a capping agent has been recently investigated by our research team.[7] As far as could be ascertained, however, there are no reported in vivo radiographic outcomes of this response. Therefore, the purpose of this study was to assess radiographically the effect of recombinant human bone morphogenetic protein-7 on the pulpal and periapical tissues after pulpotomy in dogs' teeth.

METHODS

PREPARATION OF RHUBMP-7

Under aseptic conditions, recombinant human bone morphogenetic protein-7 (rHuBMP-7, batch no. 605BMP01, ProSpec Tany TechnoGene Ltd, Rehovat, Israel) and recombinant human-like collagen (rHuCollagen, batch no. 605COL01, ProSpec Tany TechnoGene Ltd) were solubilized in pyrogen-free water (Milli-Q Ultrapure Water Purification, Millipore, Billerica, Mass) and mixed to obtain a solution at a concentration of 2.5 µg rHuBMP-7/mg rHuCollagen. The material was lyophilized (Speed Vac SC 100, Savant Instruments, Inc, Ramsey, Minn) at 25°C for 2 hours, sterilized with ethylene oxide, and stored at -20°C until use.

OPERATIVE PROCEDURES

The experimental protocol was conducted in compliance with the specifications of the Animal Experimentation Ethics Committee of the University of São Paulo, São Paulo, Brazil and according to the ISO 7405:1997.[8]

The mandibular second, third, and fourth premolars and the maxillary second and third premolars of 6 12-month-old male and female dogs of undefined breed, coming from the same litter and weighing 8 to 10 kg, were selected for this study. A total of 60 teeth (120 roots) were assigned to 8 groups, as described in Table 1. The animals were anesthetized intravenously with 3% sodium thiopental (Thionembutal, Abbot Laboratories, Rio de Janeiro, Brazil; 30 mg/kg body weight). Supplementary anesthesia was provided when required. Throughout the duration of the procedures, the animals were maintained with isotonic saline combined with 2.5% glucose (Glicolabor Indústria Farmacêutica Ltda, Ribeirão Preto, São Paulo, Brazil).

After rubber dam placement and disinfection with 3% hydrogen peroxide and 2% chlorhexidine digluconate, coronal access was obtained using air/water cooled high-speed no. 1015 diamond burs (KG Sorensen Indústria e Comércio, São Paulo, Brazil). The burs were replaced every 4 cavity preparations to ensure cutting efficiency and avoid overheating.

Table 1. Groups, tested materials, number of teeth per group and experimental periods.

Group	Material	No. of teeth (roots)	Experimental period (days)
1	Recombinant human bone morphogenetic protein-7 plus recombinant human-like collagen	10 (20)	7
2	Recombinant human-like collagen	10 (20)	7
3	Calcium hydroxide (negative control)	5 (10)	7
4	Zinc oxide and eugenol cement (positive control)	5 (10)	7
5	Recombinant human bone morphogenetic protein-7 plus recombinant human-like collagen	10 (20)	70
6	Recombinant human-like collagen	10 (20)	70
7	Calcium hydroxide (negative control)	5 (10)	70
8	Zinc oxide and eugenol cement (positive control)	5 (10)	70

The pulp chamber was irrigated with sterile saline, and the coronal pulp was amputated at the level of the root canal entrances using sharp curettes. Hemostasis was obtained by copious irrigation of the pulp chamber with saline.

All experimental groups were tested in the same animal and were performed in alternate quadrants in a change-over system distributed at random. The materials were prepared according to the manufacturers' instructions.

The following materials were used as capping agents:

a. groups 1 and 5: 3 mg rHuBMP-7/rHuCollagen (2.5 µg rHuBMP-7/mg rHuCollagen) mixed with 0.5 mL saline;
b. groups 2 and 6: 3 mg rHuCollagen mixed with 0.5 mL saline;
c. groups 3 and 7: 0.5 g calcium hydroxide pro-analysis (Calcium Hydroxide zur Analyse, Merck, Darmstadt, Germany) mixed with 0.5 mL saline; and
d. groups 4 and 8: zinc oxide and eugenol cement (IRM, Dentsply Indústria e Comércio Ltda, Petrópolis, RJ, Brazil; 1 g zinc oxide mixed with 1 drop eugenol).

In all groups, the pulp-capping material was covered with a commercial calcium hydroxide cement layer (Dycal, Dentsply Indústria e Comércio Ltda), and the access cavity was restored with resin-modified glass ionomer cement (Vitremer, 3M/ESPE, St. Paul, Minn).

Periapical radiographs were taken before the operative procedures and 7 and 70 days postoperatively using the custom-made film-holding device for standardization of the radiographic technique in dogs described by Cordeiro et al.[9] The radiographs were taken with size 2 periapical films (Ultraspeed, Eastman Kodak Company, Rochester, NY) and

an X ray equipment (Heliodent, Siemens, New York, NY) operating at 60 kVp and 10 mA with 1-second exposure time. The exposed films were processed manually by the time/temperature method.

The radiographic examination was performed by 3 calibrated examiners (K=0.9636) who evaluated integrity of the lamina dura, presence of areas of periapical bone rarefaction, internal/external root resorption, and dentin bridge formation. Data were submitted to statistical analysis by Fisher's exact test and Bonferroni correction at a 1% significance level.

The radiographs of the teeth that had radiolucent images suggestive of periapical lesions associated with the roots were

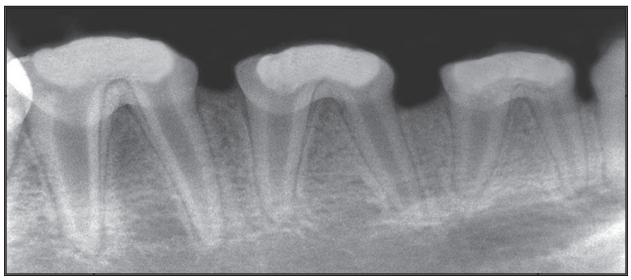

Figure 1. Group 1 (7 days) – rHuBMP-7TM /rHuCollagenTM. Periapical radiograph showing aspects of normality.

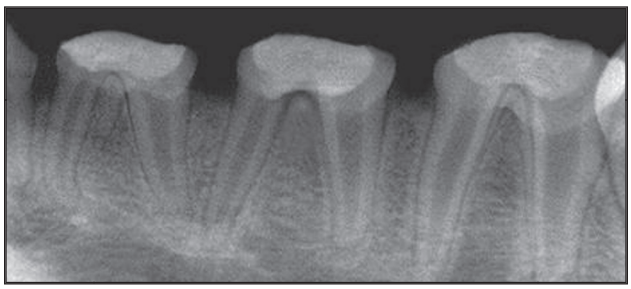

Figure 2. Group 2 (7 days) – rHuCollagenTM. Periapical radiograph showing aspects of normality.

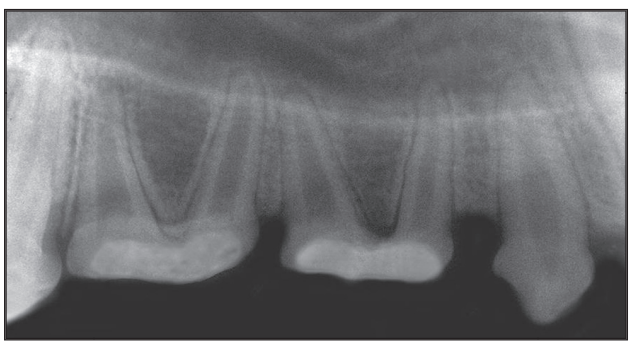

Figure 3. Group 3 (7 days) – Calcium hydroxide. Periapical radiograph showing aspects of normality.

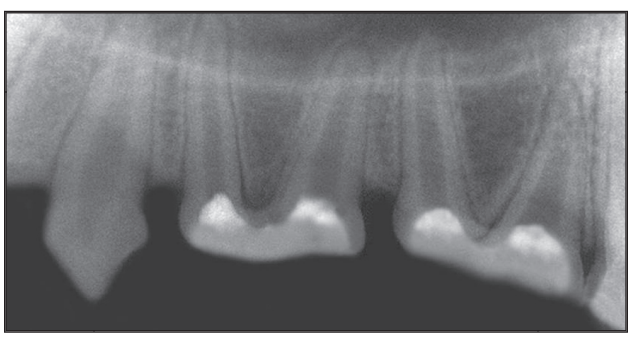

Figure 4. Group 4 (7 days) – Zinc oxide and eugenol cement. Periapical radiograph showing aspects of normality.

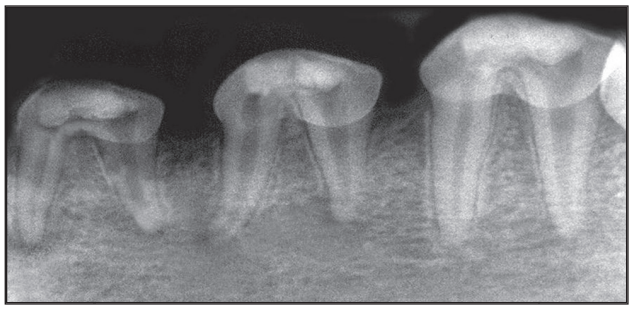

Figure 5. Group V (70 days) – rHuBMP-7TM /rHuCollagenTM. Resorption of the lamina dura and presence of radiolucid areas suggesting chronic periapical lesion.

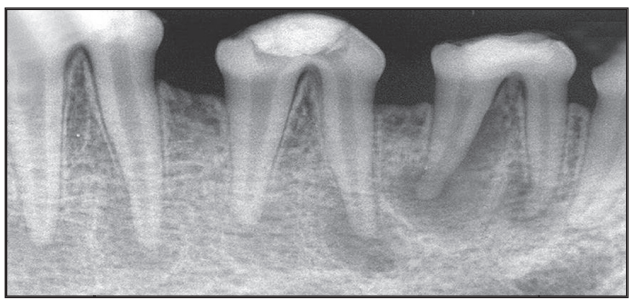

Figure 6. Group 6 (70 days) – rHuCollagenTM. Resorption of the lamina dura and presenceof radiolucid areas suggesting chronic periapical lesion.

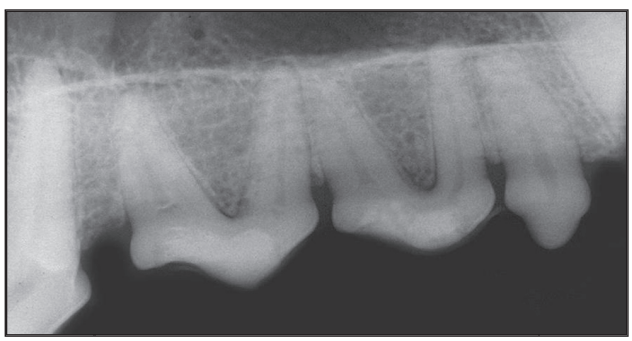

Figure 7. Group 7 (70 days) – Calcium hydroxide. Presence of dentin bridge, integrity of the lamina dura e absence of alterations on the osseous tissue.

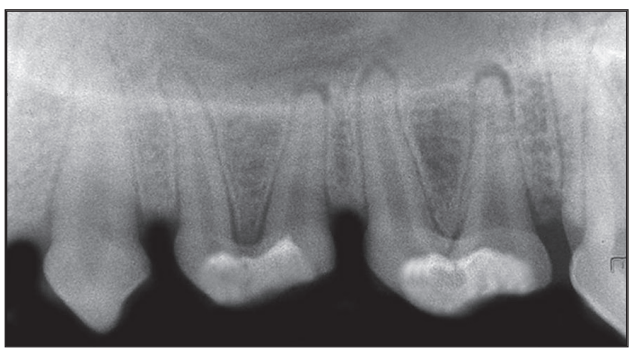

Figure 8. Group 8 (70 days) – Zinc oxide and eugenol cement. Resorption of the lamina dura and presence of radiolucid areas suggesting chronic periapical lesion.

Table 2. Results of the radiographic evaluation 70 days after pulpotomy, regarding the following parameters: integrity of the lamina dura, areas of periapical bone rarefaction, internal/external root resorption and dentin bridge formation. The values are expressed in number of roots and percentage.

	Integrity of the lamina dura		Periapical bone rarefaction		Internal root resorption		External root resorption		Dentin bridge formation	
	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)	Absent N (%)	Present N (%)
rHuBMP7/ rHuCollagen (Group 5 –20 roots)	8 (40)	12 (60)	12 (60)	8 (40)	20 (100)	0	20 (100)	0	20 (100)	0
rHuCollagen (Group 6 –20 roots)	20 (100)	0	0	20 (100)	20 (100)	0	8 (40)	12 (60)	20 (100)	0
Calcium Hydroxide (Group 7 –10 roots)	0	10 (100)	10 (100)	0	10 (100)	0	10 (100)	0	4 (40)	6 (60)
Zinc Oxide and Eugenol (Group 8 –10 roots)	6 (60)	4 (40)	4 (40)	6 (60)	10 (100)	0	7 (70)	3 (30)	10 (100)	0

digitized using an optical scanner (Scanjet 7450 c series, Hewlett-Packard, San Diego, Calif) and lesion dimensions were measured in mm^2 using Image J 1.28 u software, (National Institutes of Health, Bethesda, Md). To calibrate the software, the distance from the cusp height to the cervical margin of each tooth's distal surface was measured using a compass, and this measurement was transferred to the software. The values obtained (in mm^2) were analyzed statistically via the Kruskal-Wallis nonparametric test at a 5% significance level.

RESULTS

In the 7-day experimental period, all groups presented intact lamina dura and absence of periapical bone rarefaction, root resorption (internal or external), or dentin bridge formation. Radiographic alterations were not observed in any group (Figures 1-4).

In the 70-day experimental period, groups 5 (rHuBMP-7/ rHuCollagen), 6 (rHuCollagen), and 8 (zinc oxide and eugenol) presented discontinued lamina dura with periapical lesion formation. These findings were different from those of group 7 (calcium hydroxide; $P<.01$; Figures 5-8). External root resorption was observed only in groups 6 (rHuCollagen) and 8 (zinc oxide and eugenol), which differed significantly from the other groups ($P<.01$). Dentin bridge formation occurred only in 60% of the specimens of group 7 (calcium hydroxide) and was not observed in the other groups. Internal root resorption was not observed in any of the groups (Table 2). Areas of periapical bone rarefaction suggestive of a periapical lesion were detected in the specimens of groups 5 (rHuBMP-7/rHuCollagen), 6 (rHuCollagen), and 8 (zinc oxide and eugenol). The results of the measurements of the radiolucent areas suggestive of periapical lesions associated with the teeth in each group are given on Table 3.

There was a statistically significant difference among the groups regarding the periapical lesion size ($P<.05$). The lesions in group 6 (rHuCollagen) were significantly larger than those in groups 5 (rHuBMP-7/rHuCollagen) and 8 (zinc oxide and eugenol) ($P<.05$), which, however, did not differ significantly to each other ($P<.05$).

Table 3. Measurements of the radiolucent areas (mm2) suggestive of periapical lesion in groups 5, 6, 7 and 8 (70–day experimental period).

	Measurements of the radiolucent areas (mm2)				
Grupo 5	13.61	11.06	0	0	0
	14.74	8.4	0	0	0
	6.72	5.8	0	0	0
	4.55	2.33	0	0	0
Grupo 6	25.19	20.85	17.34	6.99	2.85
	27.82	24.53	13.24	4.32	3.85
	15.32	7.47	10.23	3.37	3.78
	13.47	5.83	10.98	2.23	2.54
Grupo 7	0	0	0	0	0
	0	0	0	0	0
Grupo 8	6.73	3.96	3.95	0	0
	5.69	4.46	6.67	0	0

DISCUSSION

Before being cleared for clinical applications, every dental material should undergo a number of tests at different levels whose results substantiate its use in human beings in a safe and effective manner. Therefore, this study evaluated radiographically the pulp and periapical tissue response after pulpotomy in dogs' teeth and the use of recombinant human bone morphogenetic protein-7 as a pulp capping agent.

Bone morphogenetic proteins have been described as promising materials for conservative pulp therapies because they have been claimed to be capable of inducing the deposition of reparative dentin without causing damage to the pulp tissue.[1,4,10-17] In the present study, however, the use of bone

morphogenetic protein-7 as a capping material after pulpotomy resulted in the formation of areas of periapical bone rarefaction and the absence of dentin bridge formation.

In all specimens of the group capped with rHuCollagen, there was discontinuity of the lamina dura and areas of periapical bone rarefaction. The lesions in this group were larger and more extensive than those in groups 5 (rHuBMP-7/rHuCollagen) and 8 (zinc oxide and eugenol). Although in the present study external root resorption was detected radiographically in 60% of the group 6 specimens. The histopathological evaluation showed the occurrence of cementum and alveolar bone resorption in all cases.[7] These biomaterials have a biological behavior similar to that of zinc oxide and eugenol cement, which is a material recognizably capable of inducing the formation of periapical lesions with inflammatory infiltrate in the pulp and periapical tissues, thickened periodontal ligament and cementum, and alveolar bone resorption.[18]

The differences between this study's findings and those of other investigations might be related to the type of carrier used (ie, rHuCollagen), which may have influenced rHuBMP-7 biocompatibility. The findings of our previous study[7] showed that, when used alone as a pulp-capping agent, collagen did not show biocompatibility. The specimens protected with this material presented extensive necrotic areas, fibril dissociation, and almost total absence of cells in the 70-day experimental period.

On the other hand, no periapical lesion was observed in the calcium hydroxide group, and dentin bridges were formed in 60% of the cases. Although the presence of dentin bridges was detected radiographically in more than half of this group's specimens, the findings of our histopathological study[7] showed dentin bridge formation in all specimens capped with calcium hydroxide. This difference may be attributed to the limitations of radiographic examination, especially in maxillary teeth. In addition, it is possible that the dentinal barrier did not have enough thickness and density to be detected radiographically in the evaluated period.[19] From a radiographic standpoint, the outcomes of the present study confirm the excellent biological properties of calcium hydroxide for use as pulp-capping agent after pulpotomy.

CONCLUSION

According to the methodology employed in this study and based on the results of the histological analysis, it may be concluded that the use of recombinant human bone morphogenetic protein-7 associated with recombinant human-like collagen for covering the radicular pulp remnant after pulpotomy did not induce the deposition of mineralized tissue, leading to the formation of radiographically visible periapical lesions.

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