Radiographic evaluation of pulpal and periapical response of dogs teeth to Emdogain® used as pulpotomy agent

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ABSTRACT

Introduction: The enamel matrix derivative (Emdogain®) is a protein extract used for periodontal healing. The objective of this study was to evaluate radiographically the pulpal and periapical response of dogs teeth after pulpotomy and use of enamel matrix derivative gel (Emdogain®). **Methods:** Pulpotomy was performed in 30 teeth (60 roots) from 3 dogs and the remaining pulp tissue was capped with the following materials: Groups 1 and 4: enamel matrix derivative gel (Emdogain®); Groups 2 and 5: calcium hydroxide; Groups 3 and 6: zinc oxide and eugenol cement. After 10 days (Groups 1-3) and 75 days (Groups 4-6) periapical radiographs were obtained and the radiographic evaluation was performed considering the integrity of the lamina dura, presence of areas of periapical bone rarefaction, root resorption (internal and external) and dentin bridge formation. **Results:** In the 10-day period, all specimens in Groups 1-3 presented absence of periapical bone rarefaction, absence of root resorption (internal and external) and absence of dentin bridge formation. In the 75-day period, Groups 4-6 did not present dentin bridge formation in any specimen. Periapical bone rarefaction areas were observed in 100% of the roots in Group 4, 62,5% of the roots in Group 6 and in 25% of the roots in Groups 5. **Conclusion:** The use of enamel matrix derivative gel (Emdogain®) as a capping material after pulpotomy lead to formation of periapical lesions and did not induce deposition of mineralized tissue.

Keywords: Pulpotomy. Calcium Hydroxide. Zinc Oxide-Eugenol Cement. Dental Enamel Proteins

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Introduction

The importance of the enamel matrix derivative (EMD; Emdogain® gel, Biora AB, Malmö, Sweden) in periodontal and bone healing has been showed histologically and clinically.[1,2,3] EMD is composed of a purified acid extract of proteins isolated from porcine dental enamel matrix, mainly amelogenin (more than 95%).[4] This protein form a non-soluble aggregate which is physiologically degraded by extracellular matrix proteases to gradually release high affinity hydroxide apatite and collagen active peptides.[4,5]

EMD-driven periodontal regeneration occurs via proliferation and recruitment of cementoblasts and posterior cementogenesis. The neocementum provides the anchorage of the tooth to the alveolar bone.[5] Current research has demonstrated that amelogenin signaling is not exclusive of ameloblasts, with a direct effect on undifferentiated mesenchymal cell proliferation in others dental cell lineages.[4,5,6,7,8,9]

During odontogenesis,amelogenin regulates dental pulp cell proliferation and differentiation,[4,10] suggesting that this protein could be a potential mediator for reparative dentinogenesis in vivo. Indeed, it has been reported that EMD is able to induce reparative dentinogenesis when used as a direct pulp capping agent.[11,12,13] Reparative dentin protects the dental pulp from additional aggressive insults which could result in degenerative or inflammatory changes.[14,15]

However, no radiographic evaluation has been performed to evaluate the effects of EMD on dental pulp tissue. Therefore, the aim of this research was to evaluate the effects of EMD on dental pulp and periapical tissues of dogs teeth submitted to pulpotomy.

Methods

Operative Procedures

The experimental protocol was conducted in compliance with the specifications of the Animal Experimentation Ethics Committee and according to the ISO 7405:1997. The mandibular second, third, and fourth premolars and the maxillary second and third premolars of 3 12-month-old male and female dogs of undefined breed, coming from the same litter and weighing 10 to 15 kg, were selected for this study. For histological analysis performed in parallel study and radiographic in the present work, a total of 30 teeth (60 roots) were assigned to 6 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). 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 number 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. 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.

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

Group	Material	Number of teeth (raízes)	Experimental period (dias)
1	Emdogain® gel	6 (12)	10
2	Calcium hydroxide (negative control)	2 (4)	10
3	Zinc oxide and eugenol cement (positive control)	2 (4)	10
4	Emdogain® gel	12 (24)	75
5	Calcium hydroxide (negative control)	4 (8)	75
6	Zinc oxide and eugenol cement (positive control)	4 (8)	75

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:

» Groups 1 (10 days) and 4 (75 days): EMD gel (Emdogain®, Biora AB, Malmo, Sweden), commercially available as a gel containing 30 mg / ml of EMD in propilenogyclol alginate (PGA). The gel was disposed on the pulpal tissue and covered with a gutta-percha and wax dish.

» Groups 2 (10 days) and 5 (75 days): 0.5 g calcium hydroxide pro-analysis (Calcium Hydroxide zur Analyse, Merck, Darmstadt, Germany) mixed with 0.5 mL saline. The paste was applied on the dental pulp tissue with a curette without excessive pressure.

» Groups 3 (10 days) e 6 (75 days): zinc oxide and eugenol cement (IRM®, Dentsply Indústria e Comércio Ltda, Petrópolis, RJ, Brazil; 1 g zinc oxide mixed with 1 drop of eugenol). The paste was applied on the dental pulp tissue with a curette without excessive pressure.

In all groups, the pulp-capping material was covered with gutta-percha under glass ionomer cement (Vidrion®, S.S. White Artigos Dentários, Rio de Janeiro, Brazil), and the access cavity was restored with amalgam.Periapical radiographs were taken prior to the operative procedures and 10 and 75 days postoperatively using the custom-made film-holding device for standardization of the radiographic technique in dogs described by Cordeiro et al.[16] 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= 0,928) who evaluated the integrity of the lamina dura, presence of areas of periapical bone rarefaction, internal / external root resorption, and dentin bridge formation. Data were submitted for statistical analysis by Fisher's exact test at a 5% significance level.

The radiographs of the teeth that had radiolucent images suggestive of periapical lesions associated with the roots were digitized using an optical scanner (Scanjet 7450 c series, Hewlett-Packard, San Diego, CA) and lesion dimensions were measured in mm^2 using Image J 1.28u software National Institutes of Health, Bethesda, MD), as previously described[17]. To calibrate the software, the distance from the mesial to distal face at the enamel-cementum junction of each tooth 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

At 10-days experimental time, Groups 1 (Emdogain® gel), 2 (Calcium hydroxide) and 3 (Zinc oxide and eugenol) presented lamina dura, absence of periapical bone resorption, external and internal resorption, and absence of dentinal bridge. On Group 1 (Emdogain® gel), discontinuity of the lamina dura was observed in 58% of the cases and periapical bone resorption and external root resorption in 17%. Similarly to other experimental groups, at 10-days experimental time no dentin bridge formation or internal root resorption was detected (Fig 1A).

At 75-days experimental time, Group 4 (Emdogain® gel) presented discontinuity of the lamina dura with periapical bone resorption in 100% of the cases (Fig 1B). Conversely, internal root resorption and dentin bridge formation were not detected, although external root resorption was present in 75%

of the cases. On Groups 5 (Calcium hydroxide) and 6 (Zinc oxide and Eugenol), discontinuity of lamina dura and periapical bone resorption were detected in 25% and 50% ($p < 0.05$) of the cases and external root resorption in 37.5% and 25% of the cases ($p < 0.05$), respectively. Internal root resorption and dentin bridge formation were not observed in any group. Table 2 shows the results of all groups.

Periapical bone resorption areas were measured in Groups 4 (Emdogaim® gel), 5 (Calcium hydroxide) and 6 (Zinc oxide and eugenol) (Table 3). These lesions were larger in Group 4 (Emdogaim® gel; $p < 0.05$), followed by Groups 6 (Zinc oxide and eugenol; $p < 0.05$) and 5 (Calcium hydroxide; $p < 0.05$).

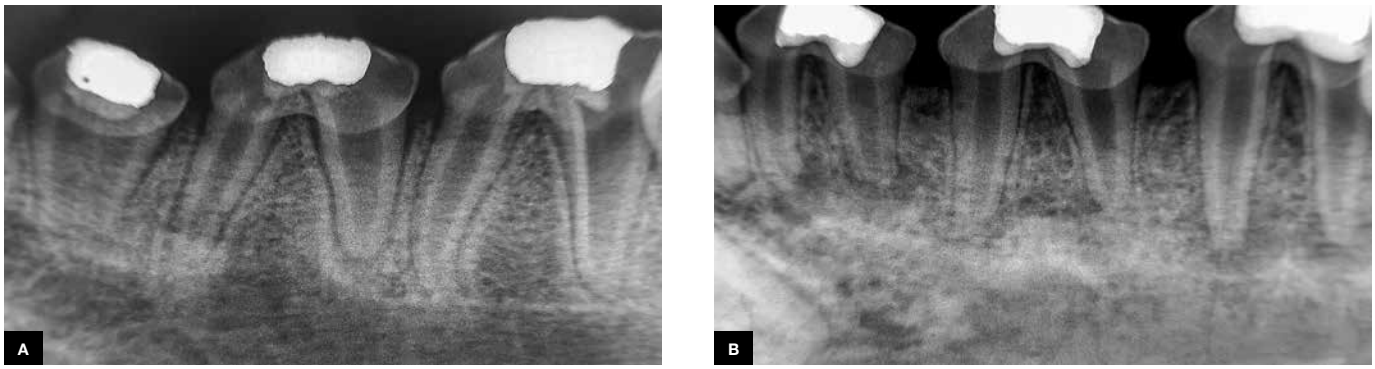

Figure 1. A) Emdogain® gel pulpotomy after 10 days. **B**) Emdogain® gel pulpotomy after 75 days.

Table 2. Results of the radiographic evaluation 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		Areas of periapical bone rarefaction		Internal root resorption		External root resorption		Dentin bridge	
	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Emdogaim® gel (10 days)	5 (42%)	7 (58%)	10 (83%)	2 (17%)	12 (100%)	0 (0)	10 (83%)	2 (17%)	12 (100%)	0 (0)
Zinc oxide and eugenol cement (10 days)	4 (100%)	0 (0)	4 (100%)	0 (0)	4 (100%)	0 (0)	4 (100%)	0 (0)	0 (100%)	0 (0)
Emdogaim® gel (75 days)	4 (100%)	0 (0)	4 (100%)	0 (0)	4 (100%)	0 (0)	4 (100%)	0 (0)	4 (100%)	0 (0)
Endogain® gel (75 dias)	24 (100%)	0 (0)	0 (0)	24 (100%)	4 (100%)	0 (0)	6 (25%)	18 (75%)	24 (100%)	0 (0)
Zinc oxide and eugenol cement (75 days)	4 (50%)	4 (50%)	4 (50%)	4 (50%)	4 (100%)	0 (0)	6 (75%)	2 (25%)	8 (100%)	0 (0)
Calcium hydroxide (75 days)	2 (25%)	6 (75%)	6 (75%)	2 (25%)	4 (100%)	0 (0)	5 (62,5%)	3 (37,5%)	8 (100%)	0 (0)

Tabela 3. Mensuração das áreas de radioluscência sugestivas de lesão periapical (mm^2), aos 75 após pulpotomia, nos Grupos 4, 5 e 6.

	G4 - Emdogain® gel	G6 - Cimento de óxido de zinco e eugenol	G5 - Hidróxido de cálcio
Mediana (Q1-Q3)	9,71 (7,22-13,04)	2,2 (0-5,39)	0 (0-0,89)

Discussion

Enamel matrix derivative (EMD) plays a role in odontogenesis and amelogenin is important for hydroxyapatite prism formation and organization during amelogenesis.[11,12] Enamel is rich in amelogenin, with is considered an enamel-specific adhesion protein.[4,18] Synergistic effect of ameloblastin and amelogenin in enamel formation and development in mutant mice has been demonstrated.[4,19,20] Hypothetically it has been suggested that EMD might be important for connective tissue repair / regeneration[15] and for dental pulp cell differentiation and dentinogenesis.[11,12]

Radiographically we could not detect dentin bridge formation in contact with EMD as demonstrated previously, aside the different methods of analysis (radiographic versus histological) or experimental model (human versus canine).[21] Periapical bone destruction was observed more frequently in Group 4 (Emdogain® gel) compared to OZE or Ca $(OH)_2$. The reason for the unsatisfactory results obtained with EMD cannot be determined radiographically but we speculate it can be attributed to the absence of antibacterial activity of this biomaterial, unlike OZE or Ca $(OH)_2$. Histological findings demonstrated that EMD recruited inflammatory cells to the dental pulp at 7 days that resulted in pulp necrosis at 70 days.[22]

Calcium hydroxide was used as negative control conforming ISO 7405:2008. Although dentinal bridge formation was not detected radiographically, the absence of periapical bone destruction indicates a favorable outcome for this material. In addition, it is possible that the dentinal barrier did not have enough thickness and density to be detected radiographically in the evaluated period.[16,23] 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

The use of enamel matrix derivative gel (Emdogain® gel) as a capping material after pulpotomy lead to formation of periapical lesions and did not induce deposition of mineralized tissue.

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