Apical Periodontium Response to Enamel Matrix Derivative as an Intracanal Medication in Rat Immature Teeth with Pulp Necrosis: Radiographic and Histologic Findings

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Abstract

Introduction: The aim of this study was to evaluate the enamel matrix derivative (EMD) biomaterial in nonvital immature teeth. **Methods:** To arrest root development, pulpectomies were performed in the lower first molars of 36 4-week-old rats; the cavities were left exposed to the oral environment for 3 weeks. Then, chemical disinfection was performed, and triple antibiotic paste (TAP) or EMD was applied in the root canals. A control group did not receive any treatment. Radiographic and histological data were evaluated after 3 and 6 weeks. **Results:** At 3 weeks, TAP promoted a milder inflammatory response and increased root lengths compared with the control group. At 6 weeks, root development and reduced periapical lesions could be observed in both test groups, mainly because of the deposition of a cementum-like tissue. EMD promoted narrower canals compared with TAP ($P < .05$). **Conclusions:** EMD deserves attention as a potential tool in the treatment of nonvital immature teeth. The ingrowth of cementum-like tissues into canal spaces favored dental wall thickness and may contribute to tooth resistance and support. *(J Endod 2012;38:449–453)*

Key Words

Apical, enamel matrix proteins, inflammation, nonvital teeth, odontogenesis, periodontium

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For years, apexification was the standard treatment for infected immature teeth. Although efficient for endodontic repair, this strategy keeps roots with wide open apices, reduced root length, and thin dental walls that are prone to fracture (1–3). Recently, other clinical approaches have been suggested concerning the preservation and stimulation of dental stem cells (4–6). Most of them are based on root canals' chemical disinfection with sodium hypochlorite and a triple antibiotic paste (7, 8). Some authors also suggest that a blood clot should be stimulated before root formation occurs (9). Case reports and preclinical studies confirm that root development can be induced in nonvital teeth, even in the absence of a blood clot (8, 10, 11). Nevertheless, the suggested therapeutic strategies show limited predictability, and the investigation of new protocols is warranted (12).

It is well documented that the secretion of enamel matrix derivative (EMD) proteins by the Hertwig epithelial sheath triggers a cascade of reactions that stimulate odontogenesis (13, 14). EMD, commercially available as Emdogain, is well recognized in periodontology for its regenerative potential (14). In the conservative treatment of dental pulp, EMD induces reparative dentin formation, also protecting pulp tissue from inflammation and degeneration (15–17). Thus, the present study aimed at evaluating the effect of EMD when applied in immature teeth with pulp necrosis.

Materials and Methods

This study was approved by Pontifical Catholic University of Rio Grande do Sul Institutional Animal Care and Use Committees (Protocol 10/00156). Fifty-four male Wistar rats were used. The animals were anesthetized intraperitoneally with ketamine and xylazine, 80 and 20 mg/kg body weight, respectively. The immature teeth pulp necrosis protocol has been detailed elsewhere (18). Briefly, endodontic access in lower first molars was performed in 4-week-old animals in order to induce periapical lesions and arrest root development in its initial stage. Dental pulps were exposed by drilling cavities on the central portion of the occlusal surface, with a 1011 HL round bur in high speed (KGSorensen, Cotia, SP, Brazil) to a depth nearly equal to the diameter of the bur (1 mm). A #25 endodontic file (Dentsply-Maillefer, Ballaigues, Switzerland) was then used to remove remnants of pulp tissue. Periapical lesion development was confirmed by radiographs taken weekly as previously reported (18, 19).

The animals were divided into 3 groups ($n = 6$ per group/period). In the control group, cavities were left open to the oral environment through the course of the experiment. For the other groups, teeth were left open to the oral environment for 3 weeks, and then root canal disinfection was performed as previously reported (18). In summary, debris were removed from the pulp chamber and cervical third of the roots using a #25 endodontic file inserted to a maximum depth of 2 mm to avoid injury to the apical portion of the canals. Root canal instrumentation was not performed. Canals were irrigated with 2.5% sodium hypochlorite followed by 0.9% sterile saline solution by using long needles, a carpule syringe, and an endodontic suction apparatus. The canals were dried and filled either with a triple antibiotic paste (TAP, metronidazole, ciprofloxacin, and minocycline, 50 mg of each

per mL in propylene glycol and macrogol; Pharma&Cia, Porto Alegre, RS, Brazil) or EMD (Emdogain; Straumann, Basel, Switzerland) using an insulin syringe.

Teeth were sealed with sterile cotton pellets and silver amalgam, and euthanasia was performed by the inhalation of isoflurane at post-operative periods of 3 and 6 weeks (18). The jaws were dissected for radiographic and histological evaluation.

Radiographic Analysis

The x-ray cylinder was adjusted in order to form a perpendicular angle with the buccal surface of the first molar. A focal distance of 30 cm was observed. The x-ray unit (Gnatus, Ribeirão Preto, SP, Brazil) was operated at 7 mA at 70 kVp with a size 2 phosphor plate and an exposure time of 0.2 seconds. A digital x-ray system (Denoptix/Gendex, Chicago, IL) was used to capture images scanned at the resolution of 300 dots per inch and saved as a TIFF format.

Image analysis was performed by 3 blinded, calibrated examiners (IC > 0.89 for all analyzed variables) using an image analysis software (Image Tool version 3.0; UTHSCSA, San Antonio, TX). For root length measurements, a line was traced from the pulp chamber floor to the most apical portion of the mesial root. Dental wall thickness at the apical third was estimated by calculating the percentage of the linear measurement of the mesial root canal width relative to the linear measurement of the entire mesial root width. The periapical lesion area at the mesial root was demarcated and measured.

Histological Analysis

The samples were fixed with buffered 10% paraformaldehyde for 24 hours. Then, the specimens were decalcified with 17% EDTA for 5 weeks, dehydrated in ascending concentrations of ethanol, and embedded in paraffin. Five-micrometer serial sections were stained with hematoxylin-eosin. For each sample, blinded examiners evaluated 3 sections in which the central portion of the roots, including the apex, was visible. A histological descriptive analysis was performed. Periapical inflammation was classified by calibrated examiners (κ = 0.79, $P < .001$) according to the following scores:

1. Absent (inflammatory cells absent or within vessels; periodontal fibers attached to cementum)
2. Mild (inflammatory cells or restricted to the apex; thickened periodontal ligament and few fibers arranged irregularly)
3. Moderate (sparsely distributed inflammatory cells not restricted to the vicinity of the apex but still presenting a more contained distribution; periodontal fibers arranged irregularly)
4. Intense (a heavy presence of inflammatory cells, widely distributed throughout the area adjacent to the root apex; severe disorganization of the periodontal support structures)

Statistical Analysis

Comparisons of radiographic and histological features among the groups were performed using 2-way analysis of variance and Bonferroni

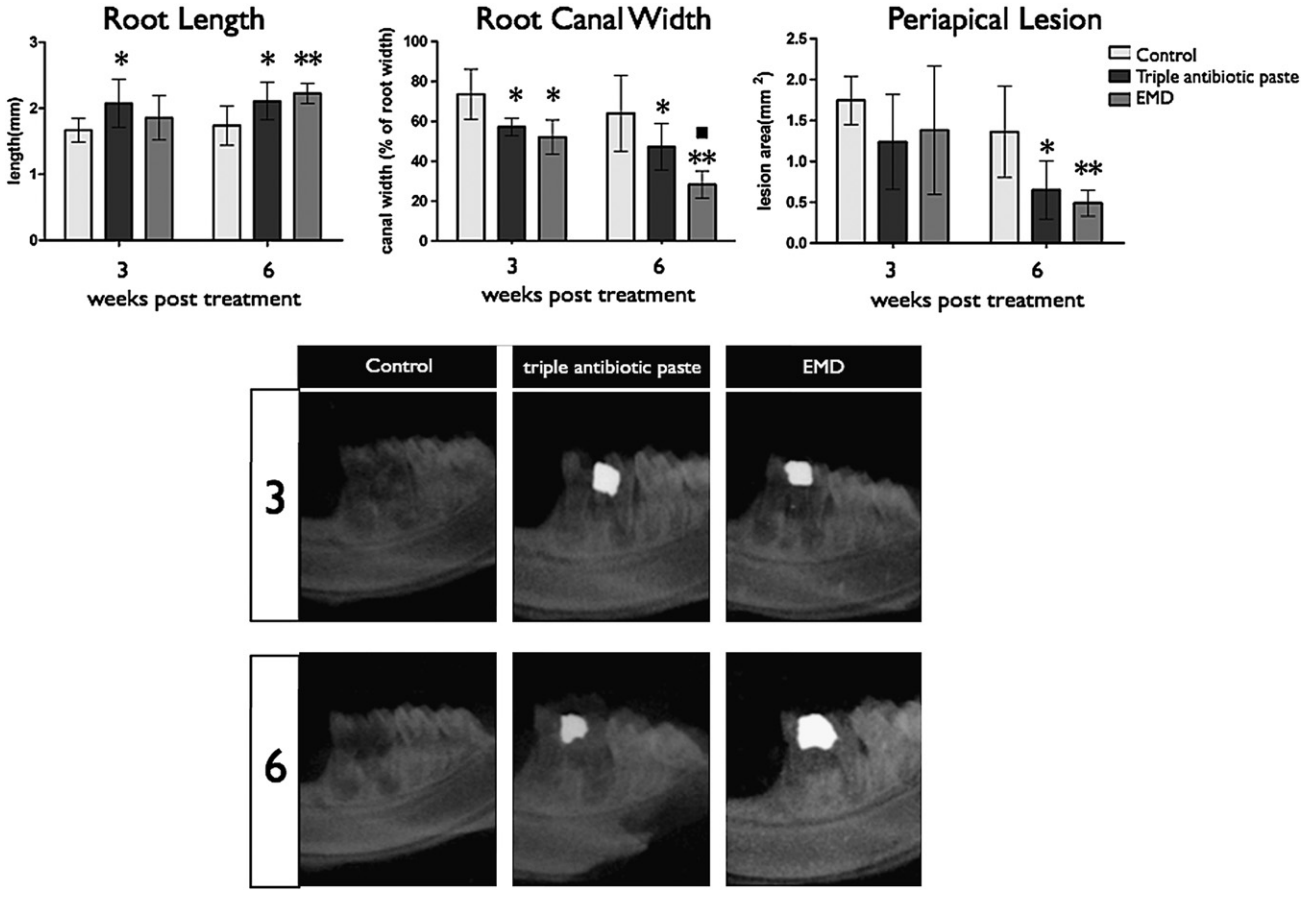

Figure 1. Radiographic analysis of root length, canal width, and periapical lesion area at 3 and 6 weeks after therapy. All parameters showed significant improvement in teeth that received treatment with EMD or TAP. Means differ significantly related to control (*$P < .05$, **$P < .01$). At the second experimental period, EMD promoted a reduced root canal width related to TAP (■$P < .05$).