Histopathological Condition of the Remaining Tissues after Endodontic Infection of Rat Immature Teeth

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Abstract

Introduction: Recently, case reports have shown that immature teeth diagnosed with necrotic pulp and periapical periodontitis can be repaired through a regenerative endodontic procedure. True regeneration depends on the presence of stem cells in the remaining vital tissues. The aim of this study was to evaluate the histologic condition of the pulp tissue, root apical papilla, and periapical tissues after inducing endodontic infection in immature rat teeth for different periods. **Methods:** This study evaluated 18 first upper rat molars (36 roots). Periapical lesions were induced and were confirmed radiographically, and the animals were divided into 3 groups according to the days of pulp exposure for endodontic infection induction: 30, 60, and 90 days. Histologic analysis was performed in 5 different areas (ie, cervical, middle, and apical root canal thirds; the apical papilla; and the periapex surrounding the apical papilla). **Results:** At 30 days, one third of the specimens still showed vital but intensely inflamed pulp tissue in the apical third and vital apical papilla with varying degrees of inflammation. After 60 days, the results were similar with respect to the apical pulp tissue and apical papilla. Completely necrotic pulp tissue in the space canal and vital apical papilla were observed in about 67% of the cases after 90 days. **Conclusions:** Vital pulp tissue was observed in the apical third until 60 days and in the vital apical papilla until 90 days of infection in a rat model. *(J Endod 2014;40:538–542)*

Key Words

Endodontic infection, immature nonvital teeth, periapical lesion, regeneration

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Recently, several clinical case reports have shown that immature teeth clinically diagnosed with nonvital pulp and periradicular periodontitis or abscess can undergo apexogenesis through a new endodontic regenerative procedure (1–4). This new protocol advocates the use of a triple antibiotic paste (metronidazole, minocycline, and ciprofloxacin) as an intracanal dressing followed by the induction of bleeding into the canal to form a blood clot (5). The blood clot is considered a scaffold and a source of growth factors for stem cells, which can mediate the regeneration and repair of tissues into the root canal (5, 6).

The pulp tissue in immature teeth with an open apex has a large volume with a rich blood supply. The open apex can provide good communication between the pulp space and the periapical tissues, making it possible for periapical disease to occur while the pulp is only partially necrotic and infected (7). Moreover, immature teeth are still in the growing phase and are likely to carry potent stem cells that enable better regeneration of tissue compared with matured tissues (5). Stem cells from the apical papilla (SCAPs), the pulp precursor, or dental pulp stem cells (DPSCs) conserved in the apex could be the precursors of new odontoblasts, which would allow either the root to undergo maturation or the rebuilding of the lost pulp tissue in the canal space (5–8).

In the dynamic environment of immature teeth, it is speculated that important types of cells and tissues from the pulp, apical papilla, and Hertwig epithelial root sheath may survive infection (8). On the other hand, hypothetically, the number of surviving cells and tissues decreases, and stem cell viability is reduced as the duration of the infection period increases (5, 8). Thus, it is possible that surviving pulp cells could be found even in those cases in which an apical lesion has been clinically and radiographically diagnosed, which would explain the apexogenesis process. Based on this assumption, the aim of this study was to evaluate the histologic condition of the remaining pulp tissue, apical papilla, and periapical tissues after exposing immature rat teeth to distinct infection periods.

Materials and Methods

Animals

The experimental protocol was approved by the Araçatuba School of Dentistry local animal ethics committee, and the animals were maintained according to the *Guide for the Care and Use of Laboratory Animals* from the National Institutes of Health (Bethesda, MD). In this study, we used a total of 18 upper first molars (36 roots) of 18 male Wistar rats weighing 160–180 g and aged 5 weeks at the beginning of the experiments. The animals were housed under standard controlled conditions and were provided a standard rat chow diet and water ad libitum.

Induction of Periapical Lesions

All rats were anesthetized by an intramuscular injection of a combination of xylazine (10 mg/kg) and ketamine (100 mg/kg). Periapical lesions were induced as described previously (9) with minor adaptations. The pulps of the right and left upper first molars of all animals were surgically exposed to the oral cavity to allow the formation of periapical lesions. This procedure was performed with high-speed rotation under constant irrigation with a ¼-sized round steel bur; the depth was equal to the bur diameter so that furcal perforation would be avoided. After exposing the pulps to the oral environment, the

animals were divided into 3 different groups based on the pulp exposure time: G1, 6 animals that were killed after 30 days; G2, 6 animals that were killed after 60 days; and G3, 6 animals that were killed after 90 days. Teeth with vital pulp, healthy apical papilla, and periapical tissues were used as controls.

Section Preparation

Periapical radiographic evaluation was performed before the preparation of each sample. In each experimental period, the teeth were dissected with the surrounding bone, immediately immersed in 10% buffered formalin solution for 48 hours, and demineralized in 10% EDTA (pH = 7.0) for 6 weeks. After rinsing for 24 hours in running water, the decalcified teeth were dehydrated, embedded in paraffin, sectioned serially at 6 μm in a mesiodistal plane, and stained with hematoxylin-eosin.

Histologic Analysis

The histologic analysis was performed by 2 observers blinded to treatment allocation, and the assessment of the tissue condition was performed in 5 different areas (ie, root cervical third, root middle third, root apical third, apical papilla region, and periapex surrounding the apical papilla). The following inflammatory cell response/tissue disorganization histologic score was considered: 0, none or a few scattered inflammatory cells present in the pulp, apical papilla, or periapical tissues characteristic of normal tissue/normal tissues in the canal, apical papilla, or periapex; 1, slight inflammatory cell infiltrate with polymorphonuclear leukocytes (PMNs) or mononuclear leukocytes (MNLs) involving the radicular pulp tissue, apical papilla, or periapical tissues/slight disorganization of radicular pulp tissue, apical papilla, or periapical tissues; 2, moderate inflammatory cell infiltrate with PMNs or MNLs/moderate disorganization of radicular pulp tissue, apical papilla, or periapical tissues; 3, severe inflammatory cell infiltrate with PMNs or MNLs/severe or total disorganization of radicular pulp tissue, apical papilla, or periapical tissues; and 4, tissue necrosis.

Statistical Analysis

The results are expressed as mean ± standard error of the mean of 6 animals (6 teeth/12 roots) in each experimental group. Statistical differences in each group were analyzed using the Kruskal-Wallis and Dunn multiple comparison tests. P values < .05 were considered statistically significant.

Results

Radiographic Findings

Periapical radiographic examination reveled periapical lesions in all observed period of time (Fig. 1*A–C*).

Histologic Findings

Control

The pulp tissue was completely intact, and a cell-rich zone was observed between the pulp and apical papilla (Fig. 1*E*). The apical papilla appeared to contain less blood vessels and cellular components compared with the dental pulp and the apical cell-rich zone.

30 Days

On day 30, no remaining tissue was found in all analyzed cervical thirds (12/12) or in the majority of the middle thirds (11/12). Interestingly, 1 root showed moderately inflamed pulp tissue in the middle third, which was totally preserved in the apical third (Fig. 1*A*). In the apical portion, one third of the specimens (4/12) still presented a residual pulp tissue that was characterized by an intense inflammatory cell infiltrate with PMNs and was severely disorganized (Fig. 1*B*). The apical papilla, which was preserved in all specimens, was histologically normal (1/12) or slightly (3/12), moderately (3/12), or severely (5/12) inflamed (Fig. 1*C* and *D*). The periapex surrounding the apical papilla was normal and free of any inflammatory process in 3 specimens (3/12) (Fig. 1*E*). Slight inflammation/tissue disorganization was observed in the remaining roots (2/12), and a moderate and poorly delimited inflammatory cell infiltrate with PMNs was found in the remaining cases (7/12), which were slightly or moderately disorganized in 4 or 3 cases, respectively (Fig. 1*F–H*).

60 Days

On day 60, the cervical and the middle thirds of all specimens exhibited necrotic tissue. On the other hand, as observed on day 30, remaining tissue in the apical third of the roots was found in one third (4/12) of the observed specimens, which was characterized by an intense inflammatory cell infiltrate with PMNs (Fig. 2*A* and *B*). The apical papilla was maintained in all teeth (12/12), and it had only a slight inflammatory cell infiltration with PMNs with a discrete tissue disorganization in one fourth of the roots (4/12), whereas it was moderately (3/12) or severely inflamed/disorganized (5/12) in the other cases (Fig. 2*C* and *D*). The periapical region surrounding the papilla was characterized by a slight (7/12) or moderate (5/12) inflammatory process with PMNs/tissue disorganization with a tendency for increases in both vascularity and the amount of fibers (Fig. 2*D*).

90 Days

On day 90, no pulp tissue was conserved in the cervical, middle, and apical thirds of the root canal. The apical papilla was disintegrated by the infectious process in one third (4/12) of the analyzed roots. In the specimens in which the apical papilla was preserved (8/12), a moderate (2/8) or severe (6/8) inflammatory cell infiltrate with MNLs in a severely disorganized tissue was observed (Fig. 2*E* and *F*). The periapical region surrounding the papilla was characterized by a slight (8/12), moderate (2/12), or severe (2/12) inflammatory cell infiltrate with MNLs, which were present in a small volume and were circumscribed by epithelial cells (Fig. 2*G* and *H*).

Comparison among the Periods

The results are summarized in Table 1. Statistically significant difference was just observed in the condition of the apical third remaining pulp tissue on 90 days regarding the other 2 periods ($P < .05$). No statistically significant difference was found in the periapex condition for the 3 distinct infection times ($P > .05$). Moreover, the apical papilla area was statistically more disorganized at 90 days after endodontic infection ($P < .05$).

Discussion

Recently, several clinical case reports have shown that immature permanent teeth are capable of undergoing apexogenesis despite the presence of a periapical lesion resulting from root canal infection (1–4). A sequence of clinical procedures is essential from the disinfection to the filling of the space with blood (10).

In the present study, the pulp in the apical third was preserved in one third of the cases until 60 days, and the apical papilla was preserved until 90 days, showing that the apical third and/or the papilla remained inflamed but alive even with the infection. The clinical presence of a negative response to pulpal testing with the presence of apical periodontitis is generally interpreted to indicate pulpal necrosis and infection. However, radiolucency at the periradicular region can no longer be used as a determining factor of total pulp necrosis because vital tissues can be present in pulp chambers, as observed in the present study, or