Pulp Revascularization of Immature Dog Teeth With Apical Periodontitis

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Abstract

This study examined the ability of a collagen solution to aid revascularization of necrotic-infected root canals in immature dog teeth. Sixty immature teeth from 6 dogs were infected, disinfected, and randomized into experimental groups: 1: no further treatment; 2: blood in canal; 3: collagen solution in canal, 4: collagen solution + blood, and 5: negative controls (left for natural development). Uncorrected chi-square analysis of radiographic results showed no statistical differences ($p \geq 0.05$) between experimental groups regarding healing of radiolucencies but a borderline statistical difference ($p = 0.058$) for group 1 versus group 4 for radicular thickening. Group 2 showed significantly more apical closure than group 1 ($p = 0.03$) and a borderline statistical difference ($p = 0.051$) for group 3 versus group 1. Uncorrected chi-square analysis revealed that there were no statistical differences between experimental groups for histological results. However, some roots in each of groups 1 to 4 (previously infected) showed positive histologic outcomes (thickened walls in 43.9%, apical closure in 54.9%, and new luminal tissue in 29.3%). Revascularization of disinfected immature dog root canal systems is possible. *(J Endod 2007;33:680–689)*

Key Words

Histologic outcomes, pulp revascularization, radiographic

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Pulp necrosis of an immature permanent tooth from caries or trauma arrests further development and leaves the tooth with thin, weak walls that are prone to fracture (1–3). Endodontic treatment of such a tooth is difficult because the thin walls do not forgive much mechanical instrumentation (4), and the open apex is difficult or impossible to seal with conventional methods of lateral condensation or thermoplasticized techniques (5). The traditional treatment for these teeth is long-term calcium hydroxide application to induce apexification (an apical hard tissue barrier) (6, 7). More recent treatments have used an artificial barrier of mineral trioxide aggregate (MTA) (8, 9). Both of these techniques are followed by a traditional root filling, but they do not increase the fracture resistance of the walls (2, 3). In fact, one in vitro study has raised the question that long-term calcium hydroxide therapy for apexification may leave the thin walls even more prone to fracture (10). Root-wall–strengthening methods with composite resin have been advocated (11), but they may limit the possibility of root canal retreatment if the need arises in the future (11).

Human avulsion case series (12) and controlled animal studies (13, 14) have shown radiographic and histological evidence of successful revascularization of immature permanent teeth after replantation. In this situation, the necrotic uninfected pulp acts as a scaffold for the in-growth of new tissue from the periapical area. The absence of bacteria is important for successful revascularization because the new tissue will stop at the level it meets bacteria in the canal space (13, 15). Studies to test the ability of topical antibiotics to improve revascularization outcomes in experimental avulsions (13, 14, 16) have shown that topical doxycycline and minocycline can improve radiographic and histological evidence of revascularization in immature avulsed permanent teeth.

Extrapolating from this information, it is hypothesized that disinfection of a necrotic-infected immature permanent tooth with apical periodontitis may render it to the same starting point as a necrotic uninfected avulsed immature permanent tooth (necrotic due to severance of the vascular supply but uninfected by oral bacteria). If disinfection is achieved, then revascularization should be possible for the disinfected canals, just as it is for the uninfected canals in the avulsion scenario (12, 14).

Research with topical antibiotics has shown that a combination of metronidazole, minocycline, and ciprofloxacin is effective in vitro (17) at killing common endodontic pathogens from necrotic-infected root canals. This antibiotic combination is also an effective disinfectant in vivo (18).

An empty canal space will not support in-growth of new tissue from the periapical area on its own (19, 20). Early studies on attempted revascularization used blood or blood substitutes to act as a scaffold to aid the in-growth of new tissue into the empty canal space. Most of these studies used vital teeth with complete pulpectomies followed by partial root-filling procedures (21–23) but did not show a significant benefit of inclusion of a blood clot to improve revascularization. Other studies using necrotic-infected teeth failed at attempted revascularization, primarily because of inadequate disinfection before inducing bleeding into the canal space (15).

Revascularization research has also studied collagen solutions as artificial scaffolds in the canal space. A series of studies (24–28) using bovine collagen with crystals of calcium and phosphate, as nucleation centers for hydroxyapatite formation, achieved some revascularization success; however, most of this work used teeth with vital pulps. Results with necrotic pulps disinfected with calcium hydroxide showed lower success, possibly because of calcium hydroxide-induced limited necrosis of the progenitor cells

in the periapical area that would have been instrumental in repopulating the empty canal space (29–31).

Radiographic evidence of successful revascularization of immature permanent human teeth with apical periodontitis has been described in the literature (32–34). In these cases, some form of topical antibiotics was used to disinfect the canal space before filling it with a scaffold.

The purpose of this study was to examine the ability of a collagen solution to aid revascularization of necrotic-infected immature dog root canals with apical periodontitis after disinfection with the triple antibiotic combination of metronidazole, minocycline, and ciprofloxacin.

Materials and Methods

Approval for this study was obtained from the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill. Sixty double-rooted premolar teeth in six purpose-bred mixed breed canine model dogs aged approximately 6 months were randomly divided into 5 treatment and control groups of 12 teeth each. Before any interventions, the involved teeth were radiographed using custom bite registrations (Regisil; Dentsply Caulk, Milford, DE) and radiograph paralleling devices (Dentsply Rinn, Elgin, IL). These radiographic aids were used for all subsequent radiographs to improve the alignment and position of the films and x-ray beam for direct comparison of the radiographs with minimal distortion or magnification.

The teeth were randomly divided into 48 experimental and 12 control teeth. Under general anesthesia (induction by Pentothal [Abbott Laboratories, North Chicago, IL] 13.5 mg/kg intravenously and intubation and maintenance with isoflurane [Halocarbon Laboratories, River Edge, NJ]) supplemented with local anesthesia (bupivacaine plain 0.5%, Abbott Laboratories); the pulps of all experimental teeth were mechanically exposed with a #2 round carbide bur (Brassler USA, Savannah, GA) in a high-speed hand piece (Midwest, Mondovi, WI) under nonaseptic conditions. A sterile #30 stainless steel endodontic hand file (Dentsply Maillefer, Johnson City, TN) was used to disrupt the pulp tissue in the canal spaces, without removing it from the canals. Supragingival plaque scaled from the dogs' teeth was mixed with sterile saline (0.9% sodium chloride; Hospira Inc., Lake Forest, IL). Sterile sponges (Dentsply Maillefer, Johnson City, TN) soaked in the plaque suspension were sealed temporarily in the pulp chambers of the teeth with IRM (Intermediate Restorative Material, Dentsply Caulk). The animals were given analgesics (Torbugesic 0.2 mg/kg; Butorphanol Tartrate, Fort Dodge Animal Health, Fort Dodge, IA) postoperatively following this and all operative procedures and were monitored by staff of the Department of Laboratory and Animal Medicine in the postoperative period.

The teeth were monitored radiographically by using the original custom bite registrations and paralleling devices until there was radiographic evidence of apical periodontitis (approximately 3 weeks).

All previously infected teeth were re-entered under aseptic conditions of rubber dam isolation with retractors and surface disinfection with 0.12% chlorhexidine (Alpharma USPD, Baltimore, MD) and tincture of iodine (Humco, Texarcana, TX) with the animals under general and local anesthesia. After removal of the IRM and sponge, the canals were irrigated with 10 mL of 1.25% NaOCl (sodium hypochlorite; Clorox, Oakland, CA) per tooth. No mechanical instrumentation was performed in the canals. Canals were dried with sterile paper points (Dentsply Maillefer, Tulsa, OK) and disinfected with a mixture of equal parts of metronidazole, ciprofloxacin, and minocycline (Professional Compounding Centers of America, Houston, TX) mixed with sterile saline (0.9% sodium chloride, Hospira Inc). The paste was applied to the canal spaces with a sterile lentulo spiral (Dentsply Maillefer, Johnson City, TN) in a slow speed handpiece (Midwest). The 12 teeth (24 roots) that were randomly assigned to group 1 were closed permanently at that appointment with a double coronal seal consisting of white MTA (Dentsply Tulsa Dental, Johnson City, TN) and silver amalgam (Sybraloy; Kerr Corporation, Orange, CA). The other 36 experimental teeth were closed temporarily with a sterile sponge (Dentsply Maillefer) and IRM (Dentsply Caulk) for 4 weeks to allow disinfection of the canal spaces.

After 4 weeks, under the same conditions of asepsis and general and local anesthesia, the temporary restorations and sponges were removed under rubber dam isolation from the remaining 36 experimental teeth. The antibiotic mixture was irrigated from the canals with 10 mL of 1.25% NaOCl (Clorox) and 10 mL of sterile saline (0.9% sodium chloride, Hospira Inc) per tooth. In the 24 roots randomly assigned to group 2, a sterile #30 stainless steel endodontic hand file (Dentsply Maillefer) was inserted past the canal terminus into the periapical tissues to induce bleeding to fill the canal spaces as much as possible. A type I collagen solution (rat tail type I collagen; BD Biosciences, Bedford, MA, 2.33 mg/mL in 2× phosphate-buffered saline) was placed in the 24 roots randomly assigned to group 3. The 24 roots randomly assigned to group 4 had the collagen solution placed in the canals before induction of bleeding from the periapical tissues into the canal space. All of these teeth were then closed with a double coronal seal of white MTA (Dentsply Tulsa Dental, Johnson City, TN) and silver amalgam (Sybraloy). The 12 teeth randomly assigned to group 5 were negative controls. These teeth were never operated but were left untouched to develop naturally for comparison with the experimental teeth. All of the teeth were monitored radiographically on a monthly basis for 3 months before the animals were sacrificed, and tissues were harvested for histological examination.

The animals were sacrificed under general anesthesia provided by Socumb (pentobarbital; Butler Company, Columbus, OH) at 30 mg/kg intravenously. The carotid arteries were exposed and canulated. The animals were euthanized with additional pentobarbital (Socumb, Butler Company) at a dose of 90 mg/kg intravenously. The animals were perfused with 10% buffered formalin (Fisher Scientific, Fair Lawn, NJ). The jaws with the involved teeth were resected and placed in formaldehyde (Fisher Scientific).

After removal of all soft tissue and excess hard tissue from the specimens, they were next placed in Formical (Decal Chemical Corporation, Congers, NY) for decalcification for 6 days, including one change of the solution. The specimens were subsequently decalcified in Immunocal (Decal Chemical Corporation, Tallman, NY) for 2 months, undergoing four changes of the solution over that time. On removal from the decalcification solution, the specimens were placed under a running tap water wash for 20 minutes followed by immersion in 70% ethyl alcohol. The specimens were then dehydrated through ascending gradations of ethanol and processed on a Leica TP 1020 dip n' dunk processor (Leica, Wetzlar, Germany) at 45 minutes per station in the following manner: one cycle of 70% ethanol, two cycles of 80% ethanol, two cycles of 95% ethanol, two cycles of 100% ethanol, two cycles of xylene, and two cycles of Paraplast paraffin (Kendall, Mansfield, MA) at 58°C. The tissues were then removed from the storage cassettes and embedded in paraffin and were sectioned on a Leica Jung RM 2045 microtome. Sections were made longitudinally every 5 μm through the apical foramen of the roots and placed on probe on plus slides. Tissues were stained with hematoxylin and eosin and evaluated under light microscopy at up to 10× magnification for the presence or absence of healthy, vital tissue, and revascularization pattern, if any.

Summary of Treatment and Control Groups

The groups were as follows:

Group 1: 12 teeth: infected → disinfected (Figs. 1 and 2)