The Radiopacity and Antimicrobial Properties of Different Radiopaque Double Antibiotic Pastes Used in Regenerative Endodontics

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Abstract

Introduction: We evaluated the radiopacity and antibacterial properties of various concentrations of double antibiotic paste (DAP) containing barium sulfate ($BaSO_4$) or zirconium oxide (ZrO_2) radiopaque agents. **Methods:** The radiopacity of 1, 10, and 25 mg/mL DAP containing 30% (w/v) $BaSO_4$ or ZrO_2, DAP-free radiopaque pastes, and commercially available radiopaque calcium hydroxide ($Ca[OH]_2$) were evaluated according to ISO 6876/2001 with slight modifications ($n = 6$ per group). Dentin samples ($n = 70$) infected anaerobically for 3 weeks with bacterial biofilms obtained from root canals of an immature tooth with pulpal necrosis were treated with similar experimental pastes or received no treatment ($n = 7$). After 1 week, the pastes were rinsed off, and biofilm disruption assays were conducted. To show the residual antibacterial effects, sterile dentin samples ($n = 70$) were pretreated for 1 week with the same pastes ($n = 7$). The pastes were rinsed off, and the samples were immersed in phosphate-buffered saline for 24 hours and infected anaerobically with the same bacterial biofilm mentioned earlier for 3 weeks before conducting biofilm disruption assays. Sterile dentin blocks were used in both antibacterial analyses as negative control groups ($n = 7$). Wilcoxon rank sum tests were used for statistical analyses. **Results:** No tested concentrations of $BaSO_4$ DAP or ZrO_2 DAP showed significant differences from $Ca(OH)_2$ in radiopacity. However, all tested concentrations of $BaSO_4$ DAP, ZrO_2 DAP, and $Ca(OH)_2$ exhibited significant direct antibacterial effects. ZrO_2 DAP at 1 mg/mL and $Ca(OH)_2$ did not show significant residual antibacterial effects. **Conclusions:** $BaSO_4$ DAP at 1 mg/mL provided significantly superior residual antibacterial effects and comparable radiopacity with the commercially available $Ca(OH)_2$. *(J Endod 2018;■:1–5)*

Key Words

Barium sulfate, biofilms, double antibiotic paste, endodontic regeneration, immature tooth, zirconium oxide

Significance

The present study introduced radiopaque double antibiotic pastes at control concentrations that showed direct and residual antibacterial effects against a clinical isolate obtained from an immature tooth with necrotic pulp.

The introduction of endodontic regeneration procedures has rejuvenated the use of various antibiotic mixtures as intracanal medicaments. Recent *in vitro* studies have found that intracanal antibiotic medicaments such as triple or double antibiotic paste (DAP) may offer superior root canal disinfection in comparison with traditional calcium hydroxide ($Ca[OH]_2$) intracanal medicament (1, 2). However, unlike $Ca(OH)_2$, clinically used concentrations (500–1000 mg/mL) of these antibiotic mixtures were found to exert cytotoxic effects on stem cells from apical papillae (3, 4), dental pulp stem cells (5), and dental pulp fibroblasts (6). Therefore, evidence-based recommendations suggested the use of $Ca(OH)_2$ or low concentrations of DAP or triple antibiotic paste ranging between 0.1–1 mg/mL in an attempt to provide an efficient antimicrobial medicament without jeopardizing the fate of pluripotent stem cells within the root canal system (7).

The inability of these antibiotic medicaments to be visualized radiographically may offer a challenging aspect to clinicians in terms of insuring satisfactory application of the medicaments. Indeed, the use of radiopaque intracanal medicaments can be of particular importance during endodontic regeneration because of the presence of large blunderbuss apices of immature teeth with necrotic pulps. Therefore, over- or underapplication of nonradiopaque antibiotic medicaments can easily go unnoticed, which may lead to suboptimal root canal disinfection during regenerative endodontics. Radiopaque components are usually added to endodontic materials (cements, pastes, sealers, and obturating materials) to provide radiopacity that can help in determining the exact location of the root canal material in the root canal system as well as improving the ability of the material to localize anatomic structures within the root canal system. The radiocontrast agents commonly used in endodontic materials are insoluble salts of heavy metals such as barium, zirconium, and bismuth. The aim of this study was to

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introduce 2 types of radiopaque DAPs and to investigate the direct and residual antibacterial effects of these radiopaque antibiotic medicaments.

Materials and Methods

Preparation of Radiopaque DAP

Two radiopaque materials, barium sulfate ($BaSO_4$) and zirconium oxide (ZrO_2), were selected for this study. Various concentrations of DAP containing the 2 radiopaque agents were prepared as described in previous studies (8, 9) with a modification to incorporate the radiopaque materials. In summary, 10, 100, and 250 mg of equal portions of metronidazole and ciprofloxacin (Champs Pharmacy, San Antonio, TX) were independently dissolved in 10 mL sterile water to form 1-, 10-, and 25-mg/mL DAP solutions, respectively. Then, 3 g barium sulfate (Reagent Plus; Sigma-Aldrich, St Louis, MO) or zirconium oxide (5 μm powder, Sigma-Aldrich) was gradually incorporated into each DAP solution with vigorous stirring to form a 30% radiopaque DAP slurry composed of 30% (w/v) of each radiopaque material. Thereafter, 0.7 g methylcellulose powder (Methocel 60 HG, Sigma-Aldrich) was gradually dissolved into each DAP slurry at room temperature to create a pasty consistency of DAP. Finally, each radiopaque DAP was centrifuged for 15 minutes at 7000 rpm to form a bubble-free homogenous injectable paste with 1, 10, and 25 mg/mL DAP ($BaSO_4$ DAP or ZrO_2 DAP). Furthermore, DAP-free radiopaque placebo pastes were also prepared as described previously. The percentage of radiopaque agents (30% w/v) was selected based on pilot studies that examined 20%–40% of both radiopaque agents in 5% increments compared with a commercially available radiopaque $Ca(OH)_2$ intracanal medicament (UltraCal XS; Ultradent, South Jordan, UT).

Assessment of Radiopacity

The radiopacity of various concentrations of $BaSO_4$ DAP (1, 10, and 25 mg/mL), ZrO_2 DAP (1, 10, and 25 mg/mL), the 2 DAP-free placebo pastes, and the commercially available radiopaque $Ca(OH)_2$ medicament (UltraCal XS) were evaluated according to ISO 6876:2012 (10) with slight modifications. Briefly, disk-shaped plastic molds (with an internal diameter of 1 and 10 mm in thickness) were positioned on occlusal radiograph films (Insight-Kodak Comp, Rochester, NY) and filled with the tested pastes (n = 6). Radiographs of the pastes along with an aluminum step wedge with variable thickness (from 1–5 mm in 1-mm increments) were taken using a single-phase dental X-ray unit (Heliodent DS; Sirona Dental, Inc, Charlotte, NC) with 65 kV and a distance of 30 cm. The radiographic films were processed using an automatic processor. Finally, the densities of the image of the pastes were compared with that of the different thickness of the aluminum step wedge using a densitometer (X-Rite model 301; X-Rite, Grand Rapids, MI), and the radiopacity equivalent of each sample was expressed in millimeters of aluminum.

Dentin Sample Preparation

Extracted intact human teeth were used to prepare radicular dentin samples ($4 \times 4 \times 2\ mm^3$) according to a standardized protocol. Briefly, a low-speed diamond saw under constant water irrigation was used to obtain dentin samples with the standardized dimensions. The samples were polished sequentially using abrasive papers (500–2400 grit; Struers, Cleveland, OH) and a Roto Pol 31 polishing unit (Struers). The samples were sequentially irrigated with 1.5% sodium hypochlorite, double distilled water, and 17% EDTA (4 minutes each) to open dentin tubules and remove the smear layer (11). All samples were gas sterilized using ethylene oxide and maintained at 100% humidity and 4°C until they were used.

Collection of Bacterial Isolate

Institutional review board approval was obtained (#1510640949) to collect a clinical bacterial isolate from an infected root canal of an immature tooth that was indicated for endodontic regenerative treatment. The selected subject was healthy and had not used antibiotics for 6 months. Both the subject and his parent signed an informed assent and consent form before collection of the bacterial isolate. The bacterial isolate was obtained according to a standardized protocol detailed in previous studies (2, 12), anaerobically incubated in brain-heart infusion broth supplemented with 5 g/L yeast extract (BHI-YE) at 37°C for 48 hours, and frozen at −80°C until use.

Direct Antibacterial Effects of Radiopaque DAP

Sterile dentin samples (n = 70) were individually inserted into wells of sterile 96-well microtiter plates (FisherBrand Fischer Scientific, Pittsburgh, PA) with the pulpal sides oriented outward. The dentin samples were infected with 10 μL of an overnight culture (1×10^5 colony-forming unit [CFU]/mL) of the biofilm bacteria obtained from an immature tooth with pulpal necrosis, and 190 μL fresh BHI-YE was added. The infected dentin samples were incubated for 3 weeks anaerobically with weekly replacement of the BHI-YE growth media. The weekly replacement of the growth media was aimed to maintain the original taxa of the clinical isolates by limiting the nutritional supply as recommended in previous publications (13, 14). Three additional dentin samples were infected with the same biofilm bacteria and viewed under scanning electron microscopy (7800F; JEOL, Peabody, MA).

After 3 weeks, the infected dentin samples were randomized into 10 experimental groups (n = 7) and treated for 1 week at 37°C with $BaSO_4$ DAP (1, 10, and 25 mg/mL), ZrO_2 DAP (1, 10, and 25 mg/mL), $BaSO_4$ placebo, ZrO_2 placebo, $Ca(OH)_2$ (UltraCal XS), or sterile water. All infected dentin samples were treated for 1 week at 37°C and 100% humidity. A bacteria-free sterile dentin group (n = 7) was also used in this experiment as a negative control group to exclude the presence of bacterial contamination though the course of the experiment. The dentin samples in the negative control group received fresh BHI-YE and were incubated anaerobically with the rest of the samples for 3 weeks with weekly replacement of BHI-YE.

After treatment, each sample was gently irrigated with 3 mL sterile water for 1 minute to rinse off the treatment pastes, and each dentin sample was subjected to a biofilm disruption assay as described in earlier studies (15, 16). Briefly, each dentin sample was placed into a sterile test tube containing 2 mL sterile water. Each sample was sonicated and vortexed (30 seconds each) to dislodge the biofilms. The obtained biofilms were diluted, spiral plated on blood agar plates, and incubated anaerobically for 24 hours; CFU/mL was counted using an automated colony counter (Synbiosis, Inc, Frederick, MD).

Residual Antibacterial Effects of Radiopaque DAP

Additional sterilized dentin samples (n = 70) were placed in 96-well microtiter plates in order to be pretreated for 1 week with the same 10 experimental groups described previously. After treatment, the pastes were washed off from the dentin samples using sterile saline followed by a 1-minute irrigation with 5 mL 17% EDTA. Dentin samples were independently immersed in 200 mL sterile phosphate-buffered saline and stored at 37°C for 24 hours. After immersion, dentin samples were infected with an overnight culture of the biofilm bacteria obtained from the immature tooth with pulpal necrosis as discussed earlier (n = 7). The bacterial biofilms were grown anaerobically for 3 weeks as described earlier. Additional noninfected sterile dentin samples (n = 7) were used as a negative control throughout this experiment.

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