Evaluation of Residual Antibacterial Effect of Human Radicular Dentin Treated with Triple and Double Antibiotic Pastes

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Abstract

Introduction: The aim of this study was to investigate the residual antibacterial effect of human radicular dentin treated with various concentrations of triple antibiotic paste and double antibiotic paste (DAP). **Methods:** Sterilized dentin specimens were randomly assigned to 6 treatment groups and a no-treatment control group (n = 45 per group). For treatment groups, specimens were treated with either TAP or DAP at various concentrations (1000, 1, or 0.5 mg/mL) for 2 weeks. Then, each specimen was irrigated with 5 mL saline and incubated in phosphate-buffered solutions for 3, 7, 14, or 30 days. After that, *Enterococcus faecalis* was cultured on the specimens for 3 days. Each specimen was then transferred to a tube containing 200 μL saline, sonicated, and vortexed to detach the bacterial biofilm. The detached biofilm was spiral plated, and the number of colony-forming units was determined using an automated counting machine. **Results:** Dentin specimens treated with 1000 mg/mL TAP or DAP had a significant residual antibacterial effect up to 14 days and 30 days, respectively. No significant difference was observed between 1000 and 1 mg/mL TAP and DAP at all time points. Dentin treated with all concentrations of DAP has a significantly longer residual antibacterial effect compared with dentin treated with TAP at the same concentrations. **Conclusions:** Radicular dentin treated with TAP and DAP showed a significant residual antibacterial effect compared with untreated dentin. All concentrations of DAP showed a significantly longer residual antibacterial effect compared with the same concentrations of TAP. *(J Endod 2015;41:1081–1084)*

Key Words

Double antibiotic, endodontic regeneration, *Enterococcus faecalis*, substantivity, triple antibiotic

Intracanal applications of antibiotic medicaments have been used to eradicate endodontic pathogens during endodontic regeneration procedures. Triple antibiotic paste (TAP), a combination of metronidazole, ciprofloxacin, and minocycline, has been the most widely used intracanal medicament in endodontic regeneration (1). Double antibiotic paste (DAP), a combination of metronidazole and ciprofloxacin, has been suggested to avoid the discoloration caused by minocycline in TAP (2) and was successfully used in endodontic regeneration (3).

Previous clinical reports of endodontic regeneration have used both TAP and DAP in a concentration of approximately 1000 mg/mL (1). However, this high concentration may have adverse effects on the survival of the stem cells of dental papilla (4) and the chemical and mechanical structure of radicular dentin (5, 6). Low concentrations of both TAP and DAP (0.001–0.003 mg/mL) were found to be effective in reducing early bacterial biofilm formation of 2 commonly isolated endodontic bacteria (7). Therefore, a recent recommendation suggested the use of antibiotic mixtures in a concentration of no more than 1 mg/mL during endodontic regeneration (8).

Tetracyclines, including minocycline and doxycycline, are broad-spectrum antibiotics that have been widely used in endodontics. Radicular dentin exposed to different tetracycline-containing irrigants (ie, doxycycline in MTAD [Dentsply Tulsa Dental, Tulsa, OK] and Tetraclean [Ogna Laboratori Farmaceutici, Muggiò, Italy]) was found to have a residual antibacterial effect (9–12). Indeed, tetracycline derivatives have been reported to attach to dentin (9) and significantly bind to collagen (13). A recent study proposed that more than 85% of TAP significantly bind to radicular dentin to a depth of approximately 350 μm from the root canal surface (14). Furthermore, it has been suggested that TAP and DAP medicaments may have an indirect toxic effect on dental papilla stem cells after their removal (15), which indicates that antibiotic medicaments might have a residual antibacterial effect on radicular dentin. To the best of our knowledge, no previous studies have investigated the residual antibacterial effect of TAP and DAP on radicular dentin. Therefore, the aim of this study was to investigate and compare the residual antibacterial effect of both TAP and DAP on human radicular dentin over different time points.

Materials and Methods

Bacterial Strain and Media

Anaerobic blood agar plates (CDC; BioMerieux, Durham, NC) were used to initially grow and maintain *Enterococcus faecalis* bacteria (American Type Culture Collection 29212). Brain-heart infusion broth supplemented with 5 g/L yeast extract

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was used to grow the bacterium at 37°C in an anaerobic environment using gas-generating sachets (GasPak EZ; Becton, Dickinson and Company, Franklin Lakes, NJ) to produce the required environment.

Antibiotic Paste Preparation

For TAP, 1000 mg, 1 mg, or 0.5 mg *United States Pharmacopeia* grade antibiotic powder compounded of equal portions of metronidazole, ciprofloxacin, and minocycline (Champs Medical, San Antonio, TX) were mixed with 1 mL sterile water at room temperature to form 3 different concentrations of the antibiotic paste. For DAP, 1000 mg, 1 mg, or 0.5 mg *United States Pharmacopeia* grade antibiotic powder compounded of equal portions of metronidazole and ciprofloxacin (Champs Medical) were mixed with 1 mL sterile water to form the same concentrations of the antibiotic paste.

Human Dentin Specimen Preparation and Treatment

Intact human permanent premolars were collected and stored in 0.1% thymol solution at 4°C after obtaining Indiana University Institutional Review Board approval (Institutional Review Board 1303010841). The crowns were cut off using a water-cooled diamond saw, and each root was divided longitudinally into 2 halves. Each root half was used to prepare dentin slabs with the dimensions of $4 \times 4 \times 1$ ($16\ mm^3$). The pulpal side of the dentin specimen was sequentially polished with 500-, 1200-, and 2400-grit silicon carbide abrasive papers using a Struers Rotopol 31/Rotoforce 4 polishing unit (Struers, Cleveland, OH). Specimens were then sonicated for 9 minutes and washed for another 9 minutes under running water. All specimens were kept in water throughout the procedure to avoid dehydration.

A total of 315 specimens were randomly assigned into 6 treatment groups and a no-treatment control group ($n = 45$ per group). The specimens were sterilized in ethylene oxide, and each specimen was placed inside 1 well of a sterile 96 well plate with the pulp surface facing outward. For treatment groups, specimens were treated with 200 μL TAP or DAP at various concentrations (1000, 1, or 0.5 mg/mL) for 2 weeks at 37°C and 100% humidity. Thus, the pulpal surface of each specimen was covered with an approximately 0.55-mm layer of antibiotic. For the no-treatment group, the specimens were kept in 200 μL saline for 2 weeks under the same conditions.

At the end of the treatment period, the specimens were irrigated for 1 minute with 5 mL sterile saline to remove the antibiotic medicaments and were blotted dry with sterile gauze. The 45 dentin specimens from each group were then randomized into 5 subgroups ($n = 9$) and tested for a residual antibacterial effect either immediately or after 3, 7, 14, or 30 days of antibiotics removal. Dentin specimens were immersed in 200 μL phosphate-buffered saline (PBS) at 37°C until the allocated time of antibacterial testing.

E. faecalis Growth on Treated Root Specimens

After PBS immersion, the specimens from each subgroup were transferred into a new well of a sterile 96-well plate. Then, 190 μL fresh brain-heart infusion broth supplemented with 5 g/L yeast extract growth media and 10 μL of an overnight *E. faecalis* culture (10^6 colony-forming units [CFU/mL]) were added to each well and incubated anaerobically for 72 hours at 37°C. After that, the culture media was removed, and each dentin specimen was gently washed twice with sterile saline to remove unattached bacteria. The presence of bacterial biofilm was confirmed using a light microscope. Each dentin specimen was then transferred to a new plastic test tube containing 200 μL sterile saline. The tubes were sonicated for 20 seconds and vortexed for 30 seconds to detach biofilm cells. Sonication for a long time can break the bacterial cell wall. However, a previous report has shown that sonication of root dentin using saline as irrigant for 1 minute does not have a significant reduction in the CFU/mL of *E. faecalis* bacteria compared with a control group (16). Therefore, sonication for 20 seconds is not expected to produce cell lysis. The detached biofilm cells were diluted in saline and spirally plated on blood agar plates (CDC, BioMerieux). The plates were incubated for 48 hours in 5% CO_2 at 37°C, and the number of CFUs/mL was determined using an automated colony counter (Synbiosis, Inc, Frederick, MD).

Statistical Analysis

Data were analyzed using a linear mixed model analysis of variance and multiple pairwise comparisons to test the effect of treatment type and post treatment time in PBS on biofilm growth. The significance level was set at 0.05.

Results

The mixed linear model indicated significant effects of treatment type, post-treatment time in PBS, and their interaction on the residual antibacterial properties of radicular dentin ($P < .05$). All dentin specimens treated with antibiotics showed a gradual reduction in residual antibacterial effect over time ($P < .05$). Figure 1 shows that dentin treated with 1000 mg/mL TAP demonstrated a significant residual antibacterial effect compared with the untreated control group in up to 14 days of post-treatment ($P < .05$). Dentin treated with 0.5 or 1 mg/mL TAP showed a significant residual antibacterial effect compared with the untreated control group up to 7 days of post-treatment ($P < .05$). Dentin specimens treated with 1000 mg/mL DAP showed a significant residual antibacterial effect compared with the untreated control group up to 30 days ($P < .05$). Dentin specimens treated with 0.5 or 1 mg/mL DAP exhibited a significant residual antibacterial effect compared with the untreated control group up to 14 days ($P < .05$).

Dentin treated with 1 mg/mL TAP or DAP showed no significant difference in the residual antibacterial effect compared with dentin treated with 0.5 and 1000 mg/mL TAP or DAP, respectively, at all time points (Figs. 2 and 3). Dentin treated with 0.5 mg/mL TAP had a significantly lower residual antibacterial effect compared with dentin

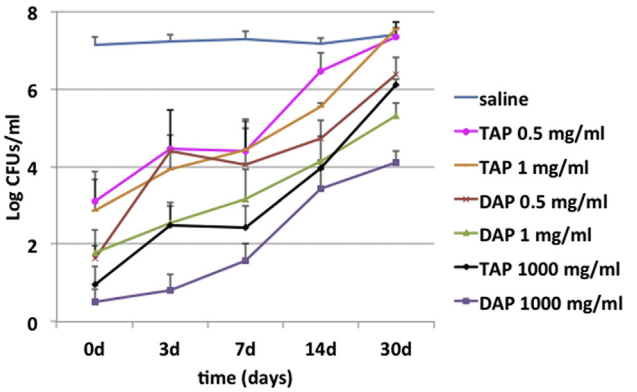

Figure 1. The residual antibacterial properties of different antibiotic medicaments and concentrations represented as the mean of the log CFU/mL over time. All dentin specimens treated with antibiotics showed a significant gradual reduction in the residual antibacterial effect over time ($P < .05$). Dentin treated with 1000 mg/mL TAP or DAP showed a significant residual antibacterial effect compared with the untreated control group in up to 14 and 30 days, respectively. Dentin treated with 0.5 and 1 mg/mL TAP or DAP showed a significant residual antibacterial effect compared with the untreated control group up to 7 and 14 days, respectively.