Spectrophotometric Analysis of Crown Discoloration Induced by Various Antibiotic Pastes Used in Revascularization

Merve Akcay, DDS, PhD, Hakan Arslan, DDS, PhD,† Bilal Yasa, DDS, PhD,‡ Fevzi Kavrık, DDS,* and Elif Yasa, DDS, PhD‖*

Abstract

Introduction: Antibiotic pastes are used for disinfection in regenerative endodontic procedures. This study evaluated the crown discoloration induced by various antibiotic pastes including the mixture of metronidazole and ciprofloxacin with minocycline, doxycycline, amoxicillin, or cefaclor. **Methods:** Seventy extracted bovine incisors were sectioned to obtain a standardized root length of 10 mm above the facial cementoenamel junction. After pulp tissue removal, irrigation with sodium hypochlorite and the placement of temporary filling material and cotton pellet were performed from the apical aspect. The specimens were then randomly divided into 7 groups (n = 10 for each group), and each group received the following antibiotic paste fillings: no filling (control group), calcium hydroxide, double antibiotic paste (DAP), triple antibiotic paste (TAP) with minocycline, TAP with doxycycline, TAP with amoxicillin, and TAP with cefaclor. Spectrophotometric readings were obtained on the buccal surfaces of the crown on day 1 to week 3 after filling, and the ΔE value was calculated. Data were analyzed with 2-way analysis of variance and the Tukey post hoc tests (P = .05), and the human perceptibility threshold was set to 3.7. **Results:** TAP with minocycline, doxycycline, and cefaclor induced more coronal discoloration compared with the control group (P < .05). The control, calcium hydroxide, and DAP groups showed no color changes exceeding the perceptibility threshold at all time points. **Conclusions:** The results indicated that all antibiotic pastes, except DAP, induced crown discoloration. *(J Endod 2014;40:845–848)*

Key Words

Amoxicillin, calcium hydroxide, cefaclor, discoloration, doxycycline, minocycline, regenerative, triple antibiotic paste

From the Departments of *Pediatric Dentistry, †Endodontics, and ‡Restorative Dentistry, İzmir Katip Çelebi University, İzmir, Turkey; and Department of Restorative Dentistry, Sifa University, İzmir, Turkey.

Address requests for reprints to Dr Hakan Arslan, Department of Endodontics, Faculty of Dentistry, İzmir Katip Çelebi University, Izmir, 35620, Turkey. E-mail address: hakan.arslan@ikc.edu.tr

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Revascularization of necrotic pulps has become an alternative conservative treatment option to the apexification procedure for immature permanent teeth. Revascularization enables thickening of the root walls by mineralized tissue and continuing physiological root development (1, 2). One of the most important stages of the revascularization procedure is the disinfection of the root canal system. To effectively eliminate infection from the root canal system, several investigators have used antibiotic pastes (2–5).

The traditional triple antibiotic paste (TAP) consisting of ciprofloxacin, metronidazole, and minocycline was developed by Hoshino et al (1). Several reports have confirmed the good antimicrobial properties of this mixture in infected root canals (1, 6, 7). Furthermore, Gomes-Filho et al (8) investigated TAP over various experimental periods and found it to be biocompatible. Despite these positive features, several case reports have shown that minocycline causes visible crown discoloration (9, 10). Recently, antibiotic alternatives to minocycline have been proposed for use in combination with ciprofloxacin and metronidazole, including amoxicillin, cefaclor, and doxycycline (2, 4, 5, 11, 12).

No known studies have compared the effect of the different antibiotic combinations used for regenerative procedures on crown discoloration. Therefore, this study used spectrophotometric techniques to assess the coronal discoloration potential of different antibiotic combinations of ciprofloxacin and metronidazole with minocycline, amoxicillin, cefaclor, and doxycycline. The null hypothesis of this study was that the tested antibiotics would not affect the color of the crown.

Materials and Methods

The sample size was calculated considering 80% power and a significance level of 0.05 using data (effect size = 4.5) obtained from the study by Lenherr et al (13). Although according to the data of this study, 14 teeth were sufficient for the analysis (n = 2), a worst-case scenario was proposed with a 0.48 effect size. According to the worst-case scenario, the sample size was calculated as 70 considering 83% power at a significance level of 0.05. Seventy bovine maxillary incisors were collected and disinfected by immersion in 0.5% Chloramine-T solution (Merck, Darmstadt, Germany) for 48 hours. Each specimen was sectioned using a bur to obtain a standardized root length of 10 mm above the facial cementoenamel junction (CEJ). The buccolingual and mesiodistal dimensions at the CEJ and the thickness between the root canal wall and external root surface were measured using electronic digital calipers and recorded for each specimen. On the basis of the sum of these 3 measurements, teeth with similar measurements were distributed equally across the 7 groups. Statistical analysis by 1-way analysis of variance (ANOVA) confirmed no significant differences among the groups in terms of their buccolingual dimensions (P = .230), mesiodistal dimensions (P = .921), and root canal wall thickness (P = .788).

All the procedures, including the removal of pulp tissue, irrigation, placement of antibiotic pastes, and placement of cotton pellet and temporary filling material, were performed from the apical aspect to avoid disruption of the intact crown and to prevent coronal microleakage (Fig. 1*A*). The pulp tissue was removed, and the root canal was irrigated with 10 mL 5.25% sodium hypochlorite to remove any remaining pulp tissue; 10 mL 17% EDTA was added for 2 minutes followed by 10 mL distilled water to simulate clinical irrigation. After the root canal space was dried using cotton, the pulp chamber

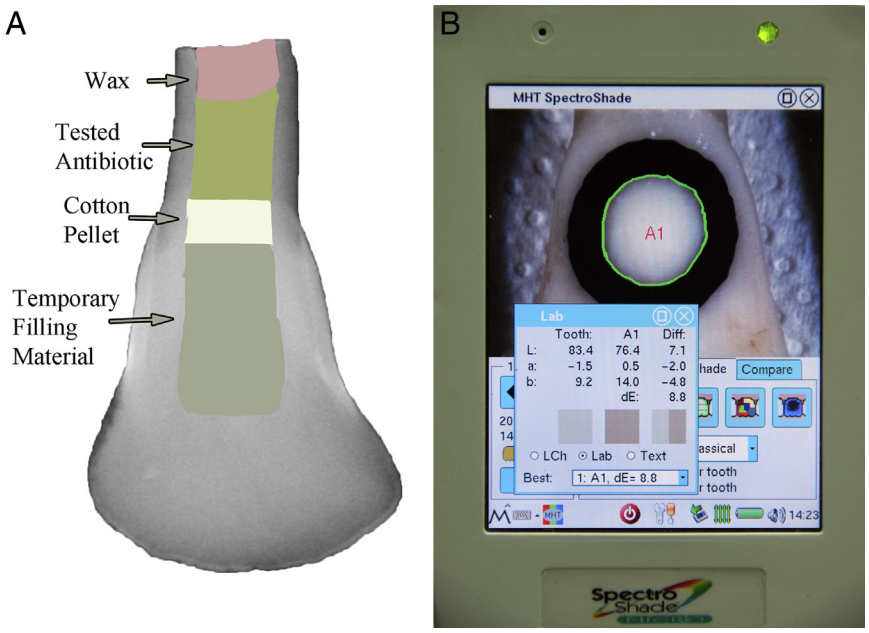

Figure 1. (*A*) A schematic presentation of apically performed procedures. Temporary filling material was placed first. A cotton pellet, tested antibiotic paste, and wax were then placed, respectively. (*B*) A representative image of the analysis of crown color of the tooth using a standardized circular strip.

was filled with temporary filling material (META Biomed Co Ltd, Cheongju, Korea) from the pulp chamber ceiling up to 2 mm below the CEJ. A cotton pellet was placed loosely on the temporary filling material up to the facial CEJ (Fig. 1*A*). The specimens were then filled according to the following groups (n = 10):

1. *Control group:* The tooth specimens in this group were left empty.
2. *Calcium hydroxide group:* The specimens in this group received calcium hydroxide (Spotdent, Izmir, Turkey) mixed with distilled water.
3. *Double antibiotic paste (DAP) group:* The specimens in this group received equal portions of metronidazole (Eczacıbası, Istanbul, Turkey) and ciprofloxacin (Biofarma, Istanbul, Turkey) mixed with distilled water (a powder/liquid ratio of 3:1).
4. *TAP with minocycline group:* The specimens in this group received equal portions of metronidazole,ciprofloxacin, and minocycline (Ratiopharm, Ulm, Germany) mixed with distilled water (at a powder/liquid ratio of 3:1).
5. *TAP with doxycycline group:* The specimens in this group received equal portions of metronidazole, ciprofloxacin, and doxycycline (Deva, Istanbul, Turkey) mixed with distilled water as mentioned previously.
6. *TAP with amoxicillin group:* The specimens in this group received amoxicillin (Bilim,Istanbul, Turkey), metronidazole, and ciprofloxacin.
7. *TAP with cefaclor group:* The specimens in this group received cefaclor (Sanovel, Istanbul, Turkey), metronidazole, and ciprofloxacin

The apical openings were sealed with sticky wax and all samples were stored at 100% humidity in an incubator at 37°C for 3 weeks.

Tooth Color Assessment

Color measurements were recorded immediately after tooth preparation, on day 1, and at weeks 1, 2, and 3 after placement of the medication. The color of each specimen was assessed by the CIE Lab system, which was defined according to the International Commission on Illumination in 1967 and referred to as CIE Lab (Commission Internationale de L'Eclairage, 1978) in L*a*b* mode by using a spectrophotometer (Spectro Shade Micro; MHT, Milan, Italy) on the buccal surfaces of the crown (Fig. 1*B*). A standardized circular strip with a diameter of 6 mm was bonded to the buccal surface of the crown 2 mm above the CEJ to ensure that color measurement was performed on the same region at every turn with a vertical angle. Before the measurement, the spectrophotometer was calibrated according to the manufacturer's instructions. The color measurements were performed 3 times at each time point on a white background, and the mean of the 3 measurements was calculated.

According to the manufacturer's recommendation, the spectrophotometer was calibrated on the white calibration tile. L* represents the value of lightness or darkness, a* represents the measurement along the red-green axis, and b* represents the measurement along the yellow-blue axis. The total color differences or distances between 2 colors (ΔE) were calculated using the following formula:

$$\Delta E* = \sqrt{\Delta L*^2 + \Delta a*^2 + \Delta b*^2}.$$

Statistical Analysis

The control and test groups were compared for differences in the baseline mean L*, a*, and b* scores by using 1-way ANOVA. The groups were compared for differences in mean ΔE at different time intervals using 2-way ANOVA. The human perceptibility threshold was set to 3.7 units to determine which differences were clinically visible (14–16).

Results

The treatment groups did not have significantly different baseline L* (P = .307), a* (P = .719), or b* (P = .697). The control, calcium hydroxide, and DAP groups did not induce color changes exceeding the perceptibility threshold at any time interval (Fig. 2*A–G*). The TAP with minocycline group induced more coronal discoloration than the other groups at all time points tested. TAP with minocycline, doxycycline, and cefaclor induced severe color changes exceeding the perceptibility