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Original Article

The discoloration effect of diluted minocycline containing triple antibiotic gel used in revascularization

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KEYWORDS
Triple antibiotic;
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Teeth discoloration

Abstract *Background/purpose:* Triple antibiotic paste (TAP) has been successfully used in revascularization procedure. However, one of the problems associated with TAP use is teeth discoloration, which is attributed to the presence of minocycline constituent. The aim of this study is to investigate the discoloration effect of different concentrations of triple (TAP) and double (DAP) antibiotics pastes on root dentine.
Materials and methods: Sterilized dentine specimens ($4 \times 4 \times 1$) were prepared, and randomly assigned to 5 groups; 1000 mg/mL of Triple antibiotic paste (TAP), 1000 mg/mL of Double antibiotic paste (DAP), 1 mg/mL of TAP in Methylcellulose gel (MTAP), 1 mg/mL of DAP in Methylcellulose gel (MDAP), and distilled water control groups ($n = 12$). The assigned treatment was applied for 14 days. The CIE L*a*b calorimetric parameters were measured for all dentine specimens using a Chroma meter. One-way ANOVA and multiple comparisons were used for statistical analyses ($p < 0.05$). ΔE for the different treatments as compared to distilled water group was calculated.
Results: TAP and MTAP groups significantly affects the L* values of the root dentine ($p < 0.05$). ΔE change was noticeable between TAP and MTAP compared to the distilled water group.
Conclusion: The incorporation of minocycline in TAP medicaments, even in low concentrations, can still provoke a noticeable tooth discoloration.

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Introduction

Endodontic regeneration, an alternative clinical approach to apexification, has received great attention in recent years because it may allow a continuation of root development.[1–3] Many case studies have shown good clinical outcomes associated with endodontic regeneration including; resolution of clinical signs and symptoms, absence of periapical infections on radiographs, continued root development and canal wall thickness of immature teeth.[4–6]

The use of antibacterial dressing to disinfect the root canal during endodontic regeneration is an essential step.[1] Triple antibiotic paste (TAP) is the most widely used medicament for root canal disinfection.[7] TAP is a combination of metronidazole, ciprofloxacin, and minocycline, which was found to be effective in root canal disinfection both *in situ* and *in vivo*[8–10] even when used in low concentration (0.3 mg/mL).[11] TAP is used clinically in high concentrations (600—1000 mg/mL) in order to form a paste consistency that can be retained inside the root canals during the disinfection step. However, the high concentration of TAP used clinically is associated with tooth discoloration that has been attributed to minocycline constituent.[12–16]

Recently, the American Association of Endodontists recommended the use of TAP in a concentration of 1—5 mg/mL. The recommended concentration shall decrease the minocycline concentration used in TAP medicament, which might solve the discoloration problem associated with the medicament. However, no previous study investigated root dentine discoloration associated with the use of diluted concentrations of TAP. Therefore; the aim of this study is to investigate root dentine discoloration caused by the use of a gel loaded with 1 mg/mL TAP antibiotics by measuring the alterations in the chromatic parameters CIE (L*, a*, b*). Our null hypothesis is that a gel loaded with (1 mg/mL) of TAP has no discoloration effect on root dentine after treatment.

Materials and methods

Antibiotic paste preparation

Five tested treatments were prepared. The first was TAP, 1000 mg of USP grade antibiotic powder compounded of equal portions of metronidazole, ciprofloxacin, and minocycline (Champs Medical, San Antonio, TX, USA) was mixed with 1 mL of distilled water at room temperature.

The second treatment was Double antibiotic paste (DAP), 1000 mg of USP grade antibiotic powder compounded of equal portions of metronidazole and ciprofloxacin (Champs Medical) was mixed with 1 mL of distilled water.

The third treatment was diluted DAP (MDAP) gel, which is consisted of 2% (w/v) Methylcellulose (MC, MW 88,000 g/mol) (Acros Organics, New Jersey, NJ, USA) and 1 mg of DAP (Champs Medical) mixed in 1 mL of distilled water at room temperature.

The fourth treatment was diluted TAP (MTAP) gel, which is consisted of 2% (w/v) MC (Acros Organics) and 1 mg of TAP (Champs Medical) mixed in 1 mL of distilled water at room temperature. The last group was distilled water, which was used as a control group. All medicaments were mixed using a magnetic stirrer (300 rpm) for 24 h to ensure proper mixing of the different pastes.

Preparation of dentine specimens

Intact human permanent premolars were taken from recollected teeth bank and stored in 0.1% thymol solution at 4 °C after extraction. The crowns were removed using a water-cooled diamond saw rotating at 300 rpm and each root was divided longitudinally into two halves. Each root half was used to prepare dentine specimens with the dimensions of $4 \times 4 \times 1$ (16 mm^3). The pulpal side of each dentine specimen was sequentially polished using 500 and 1200 grit SiC abrasive papers using a Struers Rotopol 31/ Rotoforce 4 polishing unit (Struers, Cleveland, OH, USA). Specimens were sonicated for 9 min and washed for another 9 min under running water. A total of 60 specimens were selected rand kept in water during all procedures to avoid dehydration.

Treatment of dentine

Dentine specimens were transferred into new wells of sterile 96-well plates (Thermo scientific, Wilmington, DE, USA) and randomly assigned to a total of 5 groups; 1000 mg/mL of TAP, and 1000 mg/mL of DAP, 1 mg/mL MTAP, and 1 mg/mL MDAP, and distilled water ($n = 12$). The specimens were treated with 50 μl of the different medicaments for 14 days at 37 °C and 100% humidity.

Color measurements

The CIE L*a*b calorimetric parameters was measured for all root dentine specimens using Minolta Chroma meter CR-241 (Minolta Camera, Osaka, Japan) with a light beam diameter of 0.3 mm. Samples were placed on a flat surface mold during measurement to ensure reproducibility of the position. Measurements were taken after 14 days of incubation in the medicaments. Standard D_{65} illumination was used and calibration of the device was done before use. The color was taken on 4 area of each specimen and the average was considered the color measurements for the specimen.

The CIE L*a*b system is a three dimensional system in which the L values indicate lightness on the z-axis and ranges from 0 (black) to 100 (white). The x-axis represents (a) values, which refers to red in the positive direction and green in the negative x direction, and the y-axis represents (b) values, which refer to yellow in the positive direction and blue in the negative y direction. The color difference (ΔE) between the different treatment groups and the distilled water control group was calculated using the following equation:

$$\Delta E = \sqrt{\left(L_1^* - L_2^*\right)^2 + \left(a_1^* - a_2^*\right)^2 + \left(b_1^* - b_2^*\right)^2}$$

Where ΔE is the color difference and ΔL*, Δa*, Δb* represent changes in lightness, red-green coordinate, and yellow-blue coordinate, respectively.

Statistical analysis

Data analyses were performed using Sigma Plot version 13 (Systat Software, San Jose, CA, USA). Statistical analyses were performed using ANOVA, followed by pairwise multiple comparison test. All statistical analyses were carried out at a 5% significance level. The normal distribution of data was confirmed using the Shapiro–Wilks test. The color difference value (ΔE) was considered clinically perceptible when it equals or exceeds 3.3.[17]

Results

Treating root dentine with both TAP and MTAP showed a significant effect on L* values compared with the distilled water group (Table 1). However, DAP and MDAP did not produce any significant change in L* values as compared with the distilled water group (Fig. 1). When comparing TAP and MTAP; TAP showed a significant effect on L* values compared with the MTAP group (Table 1).

Root dentine treated with TAP medicament showed a significant change in a* when compared with the distilled water group, DAP and MDAP (Table 1). All other treatments (MTAP, DAP, and MDAP) did not significantly change the a* values as compared with the distilled water group (Fig. 2). TAP showed non-significant effect on a* when compared with MTAP group (Table 1).

Root dentine treated with TAP medicament showed a significant change in b* when compared with the distilled water group, MTAP, DAP and MDAP (Table 1). All other treatments (MTAP, DAP, and MDAP) did not significantly change the b* values as compared with the distilled water group (Fig. 3).

The calculated ΔE (color difference) between the distilled water group and the other groups were 3, 3, 31, and 50 for DAP, MDAP, MTAP and TAP respectively (Table 1). A color picture of some tested dentine specimens after treatment with the different treatment groups is shown in Fig. 4.

Table 1 The calculated Δ L, Δ a, Δ b, and ΔE for the different treatments as compared to the distilled water control group.

Group	Δ L	Δ a	Δ b	ΔE
TAP	2443[a]	5[a]	94[a]	50
MTAP	984[b]	3[ab]	4[b]	31
DAP	5[c]	0[b]	2[b]	3
MDAP	1[c]	0[b]	9[b]	3
control	0[c]	0[b]	0[b]	0

Superscript letter represent the statistical significance between different groups.
TAP: Triple antibiotic paste 1000 mg/mL.
MTAP: Modified triple antibiotic paste 1 mg/mL.
DAP: Double antibiotic paste 1000 mg/mL.
MDAP: Modified double antibiotic paste 1 mg/mL.
Control: distilled water.

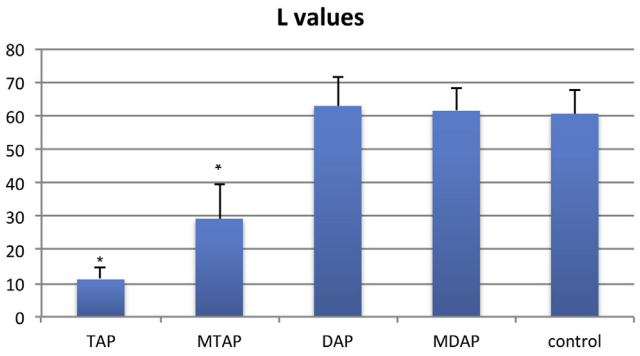

Figure 1 The L* values of root dentine after treatment with the different TAP and DAP pastes and gels as compared to distilled water control group * Statistically significant compared to the control. TAP (Triple antibiotic paste 1000 mg/mL), MTAP (Modified triple antibiotic paste 1 mg/mL), (DAP: Double antibiotic paste 1000 mg/mL), (MDAP: Modified double antibiotic paste 1 mg/mL) and control: distilled water.

Discussion

Tooth discoloration represents the most common patient complaint after endodontic regeneration treatment. The discoloration is usually caused by antibiotic medicaments used for canal disinfection[12,15,18] mainly minocycline constituent.[19] Bleaching has been advocated for the treatment of tooth discoloration by minocycline but the results are still doubtful.[12] Many methods have been used to prevent the discoloration associated with local use of minocycline in endodontic regeneration like; sealing the dentine tubules with bonding agents and the use of root canal projector during the placement of TAP inside the root canal. However, such attempts were unable to prevent the discoloration due to the high concentration of minocycline used in TAP clinically, which range between 600 and 1000 mg/mL.[12–16] The American Association of Endodontists recently recommends the use of low concentrations of TAP to a final concentration

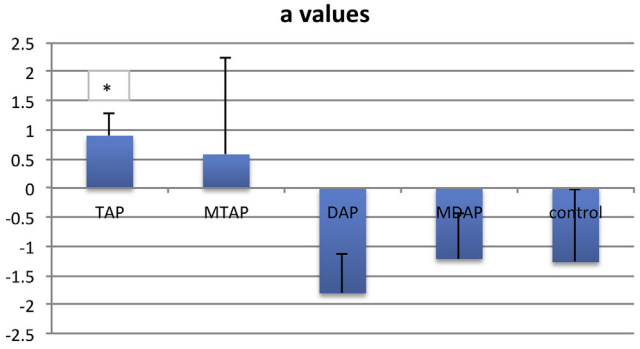

Figure 2 The a* values of root dentine after treatment with the different TAP and DAP pastes and gels as compared to distilled water control group * Statistically significant compared to the control. TAP (Triple antibiotic paste 1000 mg/mL), MTAP (Modified triple antibiotic paste 1 mg/mL), (DAP: Double antibiotic paste 1000 mg/mL), (MDAP: Modified double antibiotic paste 1 mg/mL) and control: distilled water.

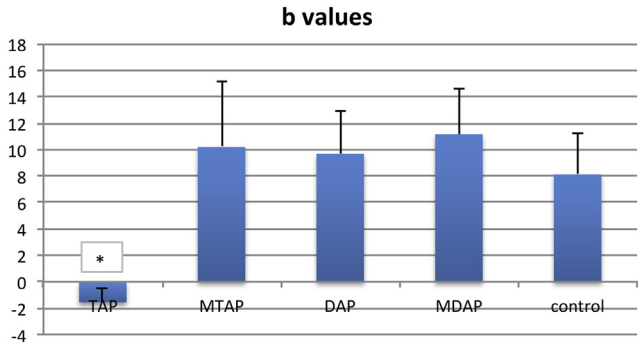

Figure 3 The b* values of root dentine after treatment with the different TAP and DAP pastes and gels as compared to distilled water control group * Statistically significant compared to the control. TAP (Triple antibiotic paste 1000 mg/mL), MTAP (Modified triple antibiotic paste 1 mg/mL), (DAP: Double antibiotic paste 1000 mg/mL), (MDAP: Modified double antibiotic paste 1 mg/mL) and control: distilled water.

of 1–5 mg/mL in order to decrease the side effects associated with it.

The results of this study showed a significant change in the root dentine L* values after treatment with TAP and MTAP medicaments as compared with the distilled water group. Although MTAP has a diluted concentration of TAP medicament, it did not prevent the discoloration associated with minocycline. Staining by minocycline and other tetracycline antibiotics has been widely described in the literature.[19–23] Minocycline antibiotics and other tetracycline antibiotics have been reported to have substantive properties as a result of its attachment to dentine and subsequent slow release from dentine.[24,25] Minocycline binds significantly to collagen, which explains its ability to discolor teeth, bone, nails, thyroid glands, and other parts of the body that are rich in collagen.[19,26] Collagen in root

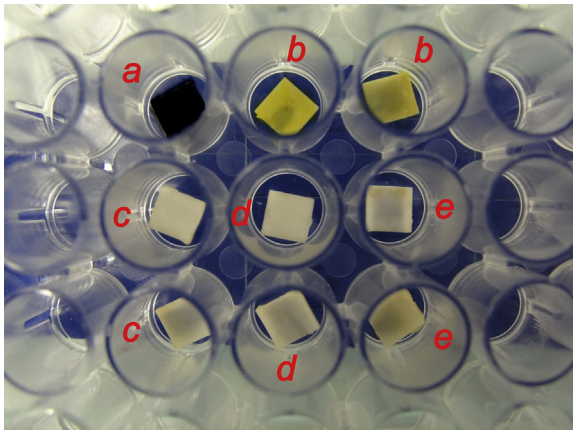

Figure 4 The color of some dentine samples treated with the different TAP and DAP pastes and gels for 14 days. a: TAP (Triple antibiotic paste 1000 mg/mL). b: MTAP (Modified triple antibiotic paste 1 mg/mL), c: control: distilled water. d: (DAP: Double antibiotic paste 1000 mg/mL), and e: (MDAP: Modified double antibiotic paste 1 mg/mL).

dentine will be exposed as a result of demineralization associated with TAP medicaments (pH = 2.8).[27] Minocycline then will be oxidized by exposure to the air or by bacterial action that will lead to degradation of the benzene ring.[26] This degradation will result in the formation of insoluble black Quinone[26] that is responsible for decreasing the L values indicating a black discoloration of root dentine.

The difference in the black discoloration between TAP and MTAP is very obvious (Fig. 4), which suggest that the final concentration of minocycline in the used medicament would significantly affect the dentine discoloration. Furthermore, higher concentration will lead to higher attachment of the minocycline to the collagen part of dentine, which will be difficult to remove from the root canal system with irrigation.

Looking at other color parameters, only TAP treatment showed significant changes of a* and b* values compared with the distilled water group. This can be explained by the formation of the black Quinone as a result of minocycline oxidation. The black Quinone has shifted the a* and b* values toward green and blue colors that are usually less light than red and yellow. Interestingly, the use of MTAP did not show a significant effect in (a) and (b) values compared with distilled water. This can be explained by the decreased concentration of minocycline in MTAP, which suggest again that the key factor in dentine discoloration is the final concentration of minocycline in the medicaments.

Human eyes is most sensitive to the lightness of the color which is presented by the L* values in the L*a*b* color system, which explains the noticeable change of ΔE observed for TAP (ΔE = 50) and MTAP (ΔE = 31) treatment groups compared with the distilled water group. The use of minocycline even in low concentration is still able to discolor teeth, and could not be a safe measure in preventing dental discoloration during endodontic regeneration treatment.

Fortunately, many other medicaments have been used successfully for disinfection of the root canal space. Those includes calcium hydroxide, DAP, and Modified TAP where minocycline is substituted with clindamycin, amoxicillin or cefaclor. However, in clinical cases that require the use of minocycline for disinfection, a low concentration (MTAP) should be used with caution and combined with other methods (i.e. sealing the dentine tubules with bonding agents and the use of root canal projector during the placement of TAP inside the root canal) to ensure a better prevention of discoloration.

Within the limitation of this *in vitro* study, minocycline-containing medicaments should not be used in endodontic regeneration when aesthetics is a major factor in the overall treatment. Further investigations should focus on finding an alternatives antibiotic for disinfecting the root canal without compromising teeth color in aesthetic zone.

Conflict of interest

None declared.

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