Comparative Analysis of Tooth Discoloration Induced by Conventional and Modified Triple Antibiotic Pastes used in Regenerative Endodontics

Abstract

Background: Success of the regenerative endodontic treatment depends upon the disinfection of the root canal system. Because almost no instrumentation is carried out, the disinfection protocol relies only on the medicaments used. The most commonly used medicament is triple antibiotic paste (TAP) composed of metronidazole, ciprofloxacin, and minocycline. The major drawback associated with the minocycline is tooth discoloration. Several antibiotics have been suggested as an alternative to minocycline. However, the tooth discoloration potential of these alternative formulations has not been documented. Aim: The present study is designed to evaluate the crown discoloration produced by different modifications of TAP. Materials and Methods: Freshly extracted human anterior teeth (n = 40) were sectioned 3 mm above and 5 mm below the cementoenamel junction. After access cavity preparation and canal enlargement, specimens were randomly divided into four groups (n = 10 for each group), and each group received the following antibiotic paste fillings: TAP with minocycline, TAP with amoxyclav, and TAP with clindamycin. Samples in control group were left empty. Images were taken with DSLR on day 0, 1, and at weeks 1, 2, and 3 at a fixed resolution. The analysis of slices was performed using the ImageJ software and mean values of pixel intensity were calculated. Statistical Analysis Used: Data were analyzed with ANOVA and Tukey HSD tests. Results: TAP with minocycline and amoxyclav induced more coronal discoloration compared with the clindamycin group (P <.001). Conclusion: Modified TAP with clindamycin did not induce clinically visible discoloration up to 3 weeks after placement.

Keywords: Discoloration, disinfection, medicament, minocycline alternative, regenerative, triple antibiotic paste

Introduction

Over the past decade, revascularization has become an alternative option to the apexification procedure for the treatment of immature permanent teeth. The procedure involves debridement of the root canal space with minimal instrumentation followed by the placement of triple antibiotic paste (TAP), which is the only source of disinfection. The traditional 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.[2-3] Gomes-Filho et al. investigated TAP over various experimental periods and found it to be biocompatible.[4] Despite these positive features, several case reports have shown that minocycline causes visible crown discoloration.[5-6] Recently, antibiotic alternatives to minocycline have been proposed for use in combination with ciprofloxacin and metronidazole, including cefaclor and doxycycline but discoloration persisted even after the substitution.[7-8]

The aim of the study is to assess the discoloration potential of modified TAP where minocycline is substituted with amoxyclav and clindamycin. These antibiotics were selected based on two criteria: (1) They do not interfere with the action of ciprofloxacin and metronidazole; (2) They have mechanism of action similar to minocycline so that the entire antimicrobial spectrum gets covered.

Materials and Method

Freshly extracted human anterior teeth (n = 40) were collected and disinfected with 0.5% chloramine-T solution for 48 hours. The teeth were sectioned 3 mm above and 5 mm below the cementoenamel junction using water-cooled diamond
points to obtain a standardized dimension. Images of all the samples were taken with DSLR camera. The images were turned into quantifiable data and the pixel intensity was measured. Statistical analysis by one-way analysis of variance (ANOVA) confirmed no significant differences among the groups in terms of pixel intensity \((P > 0.05)\). On the basis of the values obtained, teeth with similar measurements were distributed equally across the four groups. Access cavities were prepared and the canals were enlarged with Endo-Z bur, followed by copious canal irrigation with 17\% ethylene diamine tetra acetic acid and 6.0\% NaOCl to remove the smear layer. Then the specimens were divided randomly into four groups with 10 specimens in each group.

1. Control group: Tooth specimens in this group were left empty
2. TAP with minocycline group: The specimens in this group received TAP which was prepared by mixing ciprofloxacin (Sudhagar Biological and Chemicals, Chennai), metronidazole (Sudhagar Biological and Chemicals, Chennai), and minocycline (Sudhagar Biological and Chemicals, Chennai) in the ratio of 1:1:1 by weight with distilled water to get a thick paste-like consistency
3. TAP with amoxyclav group: The specimens in this group received modified triple antibiotic paste where minocycline was substituted with amoxyclav (Wockhardt, USA)
4. TAP with clindamycin group: The specimens in this group received modified TAP containing clindamycin (Sudhagar Biological and Chemicals, Chennai) instead of minocycline.

Samples were stored at 100\% humidity at 37°C for 3 weeks and in dark. Images were taken with DSLR on day 0, day 1, and at weeks 1, 2, and 3 at a fixed resolution [Figure 1]. The analysis of slices was performed by a blind observer using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).[9] The images were turned into quantifiable data and the pixel intensity was measured for each group over different time period. The mean values were then calculated [Table 1].

Statistical analysis

The groups were compared for differences in the mean value at different time intervals using one-way ANOVA. Then Tukey Honest Significant Difference (HSD) test was employed to compare all possible pairs of means at different time intervals.

Results

The TAP with minocycline group resulted in more coronal discoloration than the other groups at all-time points tested. It induced severe discoloration exceeding the perceptibility threshold from day 1 after paste placement and it increased with time. TAP with amoxyclav induced noticeable color changes from week 1. [Figure 1 and Graph 1]

The discoloration of teeth increased with time. There were statistically significant differences in the induced coronal discoloration between the initial color and the color observed on day 1 \((P < .001)\), week 1 \((P < .001)\), week 2 \((P < .001)\), and week 3 \((P < .001)\) in all the experimental groups. Despite being statistically significant, TAP with clindamycin showed the least value among all the groups and induced no visible discoloration at any tested time period [Graph 2].

Discussion

Despite the increase in the number of regenerative endodontic cases reported in the literature, there is no standardized protocol for regenerative procedures. The recommended intracanal medicaments include calcium hydroxide and TAP. However, calcification of the root canal was reported when calcium hydroxide was used as an intracanal medicament.[10] In 51\% of the published cases

| Groups                | Mean±SD Day 0 | Mean±SD Day 1 | Mean±SD Week 1 | Mean±SD Week 2 | Mean±SD Week 3 |
|-----------------------|---------------|---------------|----------------|----------------|----------------|
| Control group         | 100±0.24      | 100±0.24      | 100±0.24       | 100±0.25       | 100±0.25       |
| TAP with minocycline  | 100±0.39      | 130.49±1.52*  | 137.04±0.78*   | 139.41±1.09*   | 142.29±1.10*   |
| TAP with amoxyclav    | 100±0.42      | 110.68±0.95*  | 125.97±1.01*   | 129.71±0.71*   | 131.47±0.79*   |
| TAP with clindamycin  | 100±0.41      | 107.77±0.91*  | 109.71±0.76*   | 112.75±0.70*   | 114.45±0.82*   |

*Denotes significance at the level of 0.001. The groups were compared for differences in the mean value at different time intervals using one-way ANOVA. Then Tukey HSD (Honest Significant Difference) test was employed to compare all possible pairs of means at different time intervals. *Significance at the level of 0.001
of endodontic regeneration, TAP was used as an intracanal medicament. TAP produces a significantly greater increase in root wall thickness than calcium hydroxide.[11] Root canal infections are polymicrobial in nature. Hence, a combination is required to get rid of them. The most commonly recommended antibiotic combination is ciprofloxacin, metronidazole, and minocycline. This is because ciprofloxacin is a bactericidal broad-spectrum synthetic quinolone, which is mainly effective against Gram-negative organisms, metronidazole is a bactericidal imidazole that is highly effective against obligate anaerobes[12] and minocycline is a bacteriostatic broad-spectrum tetracycline[13] that is effective against Gram-positive organisms. One of the components of TAP is minocycline. Minocycline binds with calcium ions of the tooth via chelation to form an insoluble complex. Hence, the minocycline incorporated into the tooth matrix causes the discoloration.[14] The alternatives for minocycline with similar spectrum of action include doxycycline, amoxicillin, cefaclor and clindamycin.[15] Kim et al. reported dark discoloration of the tooth after the application of triple antibiotic paste prior to regeneration. They conducted an in-vitro experiment and concluded that among the components of the triple antibiotic paste, only minocycline caused tooth discoloration and the usage of dentin bonding agent reduced the intensity of the discoloration but did not prevent it.[5] Bleaching and coating the canal walls with dentin bonding agent was also not very much effective because 90% of TAP is retained within the canal wall, irrespective of the irrigant used.[16] Using double antibiotic paste omitting minocycline can be tried but it leads to compromised antimicrobial efficacy. Doxycycline and cefaclor can be used as substitutes, but discoloration persisted[17] even after the substitution.

Akcay et al. conducted an in-vitro study with bovine incisors which received the following medicaments: calcium hydroxide, double antibiotic paste (DAP), TAP with minocycline, TAP with doxycycline, TAP with amoxicillin, TAP with cefaclor, and assessed color changes using spectrophotometer. The results indicated that all antibiotic pastes, except DAP and calcium hydroxide, induced crown discoloration exceeding the perceptibility threshold. TAP with minocycline, doxycycline, and cefaclor induced severe color changes from day 1 and TAP with amoxicillin induced severe color changes from week 1 of evaluation.[17] In the present study, TAP with minocycline induced crown discoloration exceeding the perceptibility threshold from day 1 after paste placement, which increased with time. This is in agreement with the previous studies. TAP with amoxiclav induced noticeable color changes from week 1. Discoloration in the presence of amoxyclav is possibly caused by chromogenic precipitates.[18] TAP with clindamycin induced no visible discoloration even after 3 weeks.
Conclusion
Within the limitations of this study, it can be concluded that modified TAP with clindamycin did not induce clinically visible discoloration up to 3 weeks after placement. However, further in-vivo studies are required to evaluate the clinical efficacy.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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