History of Medicine, 2022, 8(1): 271-281

DOI: 10.17720/2409-5834.v8.1.2022.031

Assessment Of Intracoronal Bleaching's Impact On Teeth Stained By Triple Antibiotic Paste : An In Vitro Study

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Abstract

Purpose: The purpose of the study is to evaluate the effect of intracoronal bleaching agents (IBA) on discolored teeth with triple-antibiotic paste (TAP).

Methods: Twenty-six extracted maxillary incisors were discolored for 3 weeks with TAP, containing metronidazole, ciprofloxacin, and doxycycline. Following CIE L*a*b* system, colors were measured at baseline and at 1, 2, and 3 weeks. Specimens, bleached intracoronally, were randomly divided into two groups (n = 13): 35% hydrogen peroxide (HP) and 37% carbamide peroxide (CP). Color changes (ΔE) were based on the spectrophotometric measurements at baseline, 3rd, 6th, and 9th days and analyzed using analysis of variance, Bonferroni, and Student t-tests ($p < 0.05$).

Results: The discoloration increased from baseline to week 3 with significant difference ($p < 0.05$). The highest ΔE value was observed at week 3 (16.54 ± 5.90). In HP, there was no significant difference on the 6th day compared to the 3rd day ($p > 0.05$), but a significant difference on the 9th day ($p < 0.05$). In CP, ΔE was not statistically different among evaluated days. On day 9, HP had a significantly higher ΔE than CP ($p = 0.032$).

Conclusion: TAP caused clinically unacceptable ΔE, increasing over time ($\Delta E \geq 3.3$). IBA was found effective on discolored teeth. 35% Hydrogen Peroxide was more effective than Carbamide Peroxide on the 9th day.

Keywords: Anti-bacterial agent, bleaching agents, carbamide peroxide, hydrogen peroxide, tooth discoloration.

Introduction

Single-tooth discoloration can be annoying for patients and present an esthetic challenge for the clinicians[1]. The restoration of single discolored teeth has been changing from full coverage restorations, to single tooth restorations with veneers, which are more minimally invasive [2,3]. However, in such cases, clinical problems such as marginal adaptation, marginal staining, esthetic failure, debonding, and fracture are inevitable [4]. The walking bleach technique has been the most widely used since its introduction in 1961 to simplify and shorten the chair time and facilitate patient compliance [5]. The success rate will vary depending on the etiology and severity of the discoloration. The post-eruptive intrinsic local

discoloration is often caused by pulpal remnants or excessive root canal materials left in the pulp chamber[6,7]. Removing all tissues and performing proper intracoronal bleaching are generally a successful outcome for those cases[8]. The other cause is pulp degeneration caused by trauma, resulting in hemolysis of red blood cells and subsequent release of hemoglobin. Iron and hydrogen sulfide reactions produce an intense pigment iron sulfide that severely affects the tooth's color[9]. The other intrinsic factor is intracanal medicament[10]. In these cases, one of the problematic discolorations is caused by the use of tripleantibiotic paste (TAP) in Regenerative Endodontic Treatment (RET)[11,12].

RET is a biological treatment aimed at replacing affected or necrotic pulp tissue with a pulp-dentin complex to maintain the durability and functionality of the tooth[13]. With this treatment, root development is continued, root length is improved, dentin wall thickness is increased, and the closure of the apex is achieved[14]. Due to the thickness of root canal walls and to protect dentin tissue during mechanical preparation, chemical disinfection should be focused[13,15].

The latest statement of the American Association of Endodontists does not recommend any instrumentation. Thus, irrigation, and intracanal medication have become essential for disinfection and biofilm disruption[13]. Calcium hydroxide is prevalent for its antibacterial properties, but may not be effective against Enterococcus faecalis and Candida albicans, which are resistant to pH changes[16]. To overcome the limitations of calcium hydroxide, triple antibiotic paste has been proposed as an intracanal medicament. It is a mixture of Ciprofloxacin, Metronidazole, and Minocycline at a concentration of 1:1:1:1 with distilled water[17]. In regenerative studies, it is common practice to use TAP between sessions, but coronal discoloration has been reported in many cases[11,12]. Despite this one major drawback of this approach, the AAE Clinical Considerations for a Regenerative Procedure Revised (4/1/2018) guideline still recommends the use of a low concentration of TAP (1–5 mg/mL)[18].

The most common materials used for non-vital tooth bleaching are sodium perborate, carbamide peroxide (CP), and hydrogen peroxide (HP)[19]. HP and sodium perborate release oxygen-free radicals, whereas CP dissociates into HP and urea[20]. The exact bleaching mechanism is based on the oxidation reaction. Highly reactive oxygen (O) and perhydroxyl (HO2) radicals can be produced by HP (H2O2). Oxygen-free radicals are produced more in acidic environments, while highly oxidizing perhydroxyl radicals are produced more in basic environments [21]. These reactive radicals penetrate the organic matrix and the color of the dentin can be altered. However, using a higher percentage of H2O2 may increase the risk of cervical root resorption. It may occur as an inflammatory reaction by the leakage of H2O2 through the dentinal tubules into the periodontal ligament or a foreign body reaction by the protein denaturation or activation of osteoclast by a decrease in pH by H2O2 (22). Therefore, the placement of the barrier, selection, and application of bleaching material is of utmost importance [21,22]. For this reason, aggressive methods and powerful substances should be avoided. Using a higher percentage of H2O2 may increase the risk of invasive resorption [22]. Therefore, CP presents a safer alternative bleaching agent. 10% CP releases 3.5% HP, while 35% CP releases 10% HP [23].

In the literature, several methods have been described for the bleaching of teeth discolored by TAP. Intracoronal bleaching was performed with 35% HP (24), 37% CP (25), 35% HP, and

37% CP with +Nd-YAG laser irradiation [26], SP and distilled water [24,27], and SP application with heat and ultrasonic instrument [28]. However, there is no consensus on the ideal bleaching agent, and the number of bleaching studies on TAP-discolored teeth is limited. Therefore, this in vitro study aimed to evaluate the effect of intracoronal bleaching agents (IBA) on teeth discolored by TAP, addressing a much-debated question of whether a lower concentration of bleaching agent could achieve clinically acceptable esthetic results. The null hypothesis were that the effect of TAP on coronal discoloration would not be time-dependent and that there would be no significant differences between the tested bleaching agents.

Materials and Methods

Tooth Selection

Twenty-six extracted, caries-free human maxillary central and lateral incisors without any defects, pathological resorption, calcification, or existing restoration were used in this study. None of the teeth had received previous endodontic therapy. The teeth were collected after obtaining the patient's informed consent. The periodontal remnants of the extracted teeth were cleaned. The teeth were kept in 0.5% chloramine T (Merck, Darmstadt, Germany) for 1 week, and then, the teeth were stored at 4°C in distilled water and used within 1 month.

Sample Size Calculation

In the Power analysis, with d (effect size): 1.170, the minimum sample size for Power:

0.80 and α: 0.05 was defined as n = 13 for each group.

Experimental Procedure

Following Traditional Endodontic Access Cavity preparation, the root canals were irrigated using 10 mL 5% NaOCl (Septodont), 10 mL 17% EDTA (Prevest), and 10 mL distilled water with 30 gauge irrigation needle. The TAP was prepared by mixing equal powder of metronidazole (Flagly), ciprofloxacin (Cipro), and doxycycline (Actavis) with distilled water in a ratio of 1:1:1:3. After drying the root canals using paper points, TAP was placed into the root canals using a lentulo (Dentsply) and remained below the cementoenamel junction (CEJ). A small piece of the cotton pellet was placed on the CEJ and the access cavities were covered with a temporary filling material (Cavit G). Teeth were stored in distilled water within Eppendorf tubes and incubated for 3 weeks at 37°C in 100% humidity. Color measurements were made as described in the next section. The data were recorded at baseline, 1 week (1w), 2 weeks (2w), and 3 weeks (3w) after TAP was applied, respectively.

After 3w, TAP paste was removed from the root canals using copious, gentle irrigation of 20 mL of 17% EDTA with a 30 gauge endodontic needle (18). Then, the root canals were dried with paper points (Diadent). As a barrier, glass ionomer cement (Ketac Easy Molar) was applied in 3–4 mm.

Color Measurement

In this study, a composite frame was prepared to standardize the color measurements. A 6-mm diameter in a circle shape of bulk-fill composite resin (Filtek Bulkfill) was applied 2-mm coronal to CEJ on the fascial surfaces of the teeth. This way, the spectrophotometer's probe tip (Easyshade, VITA Zahnfabrick, Bad Säckingen, Germany) could be placed in the exact location. Before measuring, the spectrophotometer was calibrated. On the gray background, the measurements were repeated 3 times for each sample, and average values of color parameters were determined. The L*, a*, and b* values were recorded according to the Commission Internationale de l'Eclairage CIELAB coordinates. The color difference (ΔE) was calculated using the formula:

$\Delta E = [(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2]1/2$

Intracoronal Bleaching Steps (IBA)

The specimens were randomly divided into two groups (n = 13) according to IBA:

• Group HP - 35% HP (Opalescence Endo, Ultradent)

• Group CP - 37% CP (Whiteness Super Endo, FGM).

The bleaching agents were placed in the pulp chamber of the cavity and a cotton pellet was placed over the gel. A temporary filling material (Cavit G) was placed over the access cavity until the next session, which took place 3 days later. This procedure was repeated in each bleaching session for up to 9 days. During this process, specimens were immersed in an Eppendorf tube that contained distilled water and kept in an incubator at 37ºC and 100% humidity.

The color measurements of bleached teeth were determined on the 3rd, 6th, and 9th days. CIE L*a*b* values were recorded and color changes were computed according to the formula. In the present study, $\Delta E \leq 3.3$ was interpreted as a clinically acceptable difference.

Statistically Analysis

The parameters were assessed by Kolmogorov–Smirnov and Shapiro–Wilk tests, and it was found that the parameters were normally distributed. Student t-test was used for comparisons of parameters between two groups. Repeated measures analysis of variance was used for intragroup comparisons of the parameters and the Bonferroni test was used for post hoc comparisons. Significance was analyzed at $p < 0.05$ level.

Results

There is a statistically significant difference regarding thecolor changes (ΔE) among the evaluated periods of the 1st (1w), 2nd (2w), and 3rd weeks (3w) ($p = 0.000$). The most significant color change was observed in the 3w ($p < 0.05$) (Table 1).

	ΔE	
Time	Mean±SD	[1]p
1w	9.84±5.424[A]	
2w	13.20±5.80[B]	0.000*
3w	16.54±5.90[C]	
1w–2w[2]p	0.000*	
1w–3w[2]p	0.000*	
2w–3w[2]p	0.000*	

[1]Repeated measures analysis of variance; [2]Bonferroni test. *p<0.05. Different letters indicate statistically significant difference.

Table1. Assessment of color changes of TAP staining

In the Group HP, there was no significant difference on the 6th day when compared to the 3rd day ($p > 0.05$), but there was a significant difference on the 9th day ($p < 0.05$) (Table 2).

In Group CP, the color change was not statistically significant differed among the evaluated days ($p = 0.087$) (Table 2).

The comparison of the bleaching agents showed no statistical difference between the 3rd and 6th day ($p > 0.05$). However, on day 9, the Group HP had a significantly higher mean ΔE than the Group CP ($p = 0.032$) (Table 2).

	Group HP	Group CP	
Time	ΔE Mean±SD	ΔE Mean±SD	[1]p
3rd day	12.24±6.21[Aa]	9.81±4.26[Aa]	0.256
6th day	15.75±5.52[Aa]	11.11±6.56[Aa]	0.063
9th day	19.28±6.02[Ba]	14.14±5.46[Ab]	0.032*
[2]p	0.002*	0.087	
3rd–6th[3]p	0.170	1.000	
3rd–9th[3]p	0.003*	0.109	
6th–9th[3]p	0.005*	0.158	

[1]Student t test; [2]Repeated measures analysis of variance; [3]Bonferroni test. *p<0.05. Different capital letters in the column indicate differences between times. Different lowercase letters in rows indicate differences between groups.

Table 2. Evaluation of color changes of bleached teeth

Discussion

Teeth discoloration, which can be due to various causes, significantly contributes to esthetic failure. Particularly, a single discolored tooth creates an esthetic paradox (30). Bleaching is an excellent opportunity to treat these teeth conservatively without needing veneers or full crowns (21). However, the etiology of the case is just as crucial as the bleaching technique and materials affecting the result. In studies, it has been reported that trauma- or necrosis

induced discoloration has a better prognosis when compared to intracanal medicaments or metallic stains (7).

One of these intracanal medicaments is TAP, a mixture of three antibiotics (25). TAP has antimicrobial properties against Enterococcus faecalis and a potently influences on "regeneration and revascularization" to maintain vitality (13,31). However, tooth discoloration has been associated with TAP (13,32).

In regenerative endodontic treatment, to minimize discoloration, changing the antibiotic paste's content and using a dentin bonding agent have been recommended (19). However, our understanding of the effect of currently used bleaching materials on these discolored teeth needs to be improved. This is particularly important when patients consult the clinic with esthetic concerns about anterior tooth discoloration. While some research has been carried out on intracoronal bleaching with different agents (27), studies have yet to attempt to compare the bleaching effect of CP and HP agents on TAP discolored teeth.

Hence, the present study was conducted to evaluate and compare the bleaching effect of CP and HP on TAP discolored teeth. HP, sodium perborate, and CP are effective agents commonly used in intracoronal bleaching (6,7). However, since sodium perborate is not legal in Europe, it is not preferred anymore. While providing an effective whitening, there is a risk of weakening the mechanical properties of the dental hard tissues (33), penetration of the agent into the dentin tubules (34), and external cervical root resorption (22). The safe usage of these agents has gained in importance for the clinician.

With bleaching procedures, satisfactory whitening results can be visible after 2–4 sessions, depending on the degree of discoloration. Patients should be informed about "over-bleaching" and assess the color daily to avoid this (7). Therefore, in this study, bleaching agents were applied in three sessions and renewed every 3 days to mimic clinical conditions (9).

Spectrophotometers are the most widely used instruments for color matching. They offer the advantage of monitoring and measuring color changes by minimizing subjective errors (35). In this study, the effect of different bleaching agents on teeth colored with TAP was evaluated using a spectrophotometer. To eliminate any inconsistency during the measurements, a composite frame was applied on the buccal surface of the teeth to ensure that measurements were always taken from the exact location. The current study showed that when used in a mixture, TAP resulted in a non-acceptable coronal discoloration even after 1 week ($\Delta E > 3.3$) and this effect increased with time. The highest level of discoloration occurred on the 21st day, so the first null hypothesis is rejected. This finding agrees with the results obtained by other researchers (28,36,37).

Although the discoloration mechanism of TAP has not been fully explained, it is believed that minocycline in its content causes discoloration by chelation with calcium ions (12,25). To avoid tooth discoloration, it has been reported that minocycline, a tetracycline derivative, was replaced with doxycycline (38). In a previous study, TAP with minocycline ($\Delta E = 32.42 \pm 6.09$) showed a significantly higher ΔE compared to TAP with doxycycline ($\Delta E = 11.64$) in the 3 weeks, but both values presented unacceptable ΔE (26). This study used a mixture of ciprofloxacin, metronidazole, and doxycycline was used as a TAP paste. Similarly, in our study, the coronal discoloration increased with time, and values were found to be

unacceptable in the 3 weeks ($\Delta E = 16.54 \pm 5.90$). This finding suggested that doxycycline, a tetracycline antibiotic, was the cause of the discoloration.

In accordance with the results obtained in our study in which human teeth were used, Parikh et al. (37) reported the highest discoloration on day 21 ($\Delta E = 20.32$) in bovine teeth. Since the number and diameter of dentin tubules in the crown of bovine teeth are similar to those of human teeth (39), human and bovine teeth have been used in studies investigating tooth discoloration.

In the present study, the whitening effect increased daily, but a significant difference was only observed between the second and third sessions in the HP-treated samples. When the bleaching agents were compared, a significant difference was observed only on the day 9th. Therefore,

the second null hypothesis was rejected, which states that no significant differences would exist between the tested bleaching agents. This result is attributed to the bleaching effect increasing as the application time and concentration of HP increase. Several in vitro studies have evaluated the intracoronal bleaching of discolored teeth with TAP (24,26-28). In one of the studies using 35% HP gel (24), the authors did not notice significant differences ($p = 0.175$) in the color change, between the second (8 days) and the third (12 days) sessions. The other study reported the highest bleaching effect on the 12th day using 35% HP gel (26). However, these studies are difficult to compare directly with the present study because of differences in the number and frequency of bleaching sessions. In our study, the second bleaching session was performed on the 6th, and the third session on the 9th day. In this study, CP showed a gradual bleaching effect during the tested period. This finding may be correlated to the content of carbopol and glycerine in the composition, which provides a long-term effect of this agent by delaying the release of HP (40). The most surprising aspect of the data in this study is that 35% HP gel produced a similar bleaching effect as 35% CP to the 6th-day measurement.

As 35% CP releases 10% HP, it would be expected that 35% HP gel would have a more noticeable impact. When the studies with similar findings are reviewed, it is noteworthy that Lim et al. (41) evaluated the color change using human visual inspection in artificially blood-staining teeth. In a systematic review and meta-analysis study, it was reported that CP (35%, 37%), HP (35%), and the combination of sodium perborate with HP (3%, 30%) did not significantly differ from each other in endodontically treated discolored teeth (2).

The main limitation of this study was that it was performed in vitro conditions. The combination of blood with residual antibiotic pastes in the canal may have contributed to staining in clinical conditions. In addition, as the prognosis of bleaching is time-dependent, further research should be carried out to investigate the long-term success under both in vivo and in vitro conditions.

Conclusion

This study has demonstrated that TAP caused clinically unacceptable discoloration of teeth, which increased with time. CP and HP have a whitening effect on discolored teeth. However, the HP agent had the highest effect on the 9th day.

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