Research Article

Tooth bleaching induces changes in the vascular permeability of rat incisor pulps

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ABSTRACT: Purpose: To evaluate the inflammatory response in dental pulps of rat incisors subjected to tooth bleaching protocols with different HP concentrations and application times. **Methods:** 42 incisors from Wistar rats were submitted to tooth bleaching using concentrations of 25% or 35% HP for treatment times of 15, 30 or 45 minutes. Four non-bleached teeth were used as controls. The animals received an intravenous injection of India ink immediately after the bleaching procedure and were sacrificed 1 hour later. Six bleached teeth from each group and three controls were made transparent, and one sample from each group was processed for histological analysis. The data were statistically analyzed using Kruskal Wallis and Dunn's tests ($P \leq 0.05$). **Results:** The amount of dental pulp ink content was significantly higher in the samples that were bleached with 35% HP for 30 minutes and with both HP concentrations (25 and 35%) for 45 minutes than in the controls. For the samples bleached with the same HP concentration, the ink content was higher in samples that were bleached for 45 minutes. These results indicate that HP tooth bleaching can induce an increase in vascular permeability in rat incisors. Importantly, this increase is more dependent on the length of the bleaching procedure than on the concentration of the bleaching agent. (*Am J Dent* 2013;26:298-300).

CLINICAL SIGNIFICANCE: The cause of bleaching-related sensitivity is not well understood. Different exposure time and HP concentrations applied to enamel and dentin of different thicknesses should be tested to prevent any major vascular effects on the local pulp tissues and development of pulp sensitivity.

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Introduction

Tooth bleaching is a conservative treatment that is often requested by patients with discolored teeth. In-office bleaching is preferable to many at-home methods because it produces more immediate results.[1]

Hydrogen peroxide (HP), which is used at different concentrations, is the main active chemical component in the bleaching process.[2] Due to its low molecular weight, it can penetrate the dentin tubules and reach the pulp chamber.[3] The degradation of HP produces free radicals, which can cause the destruction of the organic and mineral components of dentin.[4,5] In addition, these products have a damaging cytopathic effect on the pulp cells,[6,7] which is proportional to the concentration of the HP.[8-10]

In vivo studies have observed the influence of HP on dental pulp tissues. Kina at al[9] did not observe damage to the pulp tissue of premolars subjected to bleaching with 38% HP for 30 minutes. However, Costa et al[10] reported necrosis in the coronal pulp and inflammatory changes within the dilated blood vessels of the radicular pulp when incisors were bleached with 38% HP for 45 minutes. The authors predicted that the differences in these results are due to variability in the thickness of enamel and dentin between the premolars and incisors studied; however, differences in HP exposure time should also be considered.

A light source can be used in tooth bleaching, but the latest gels do not require light activation. Furthermore, bleaching with light activation is no more effective than bleaching without light activation,[11] and the use of light activation can increase the vascular permeability[12] and the level of inflammatory substances in the pulp.[13] To date, studies evaluating the pulpal response to non-light-activated bleaching protocols are lacking. Therefore, this study evaluated the level of inflammatory response within dental pulps of rat incisors subjected to in-office non-light-activated tooth bleaching with different concentrations of HP and different application times.

Materials and Methods

After approval from the Ethical Committee of the Veterinary School (1858/2009), 12 adult male Wistar rats (*Rattus norvegicus albinus*) weighing an average of 180 to 200 g were anesthetized using a mixture of 2% Xylazin[a] and 10% Ketamine[a] (0.1 mL per 100 g of rat bodyweight). The animals were then fixed on a surgical table, and prophylaxis and dental isolation with a light-cured resin-based gingival barrier (Whitegold Protector[b]) were performed prior to the bleaching process.

The experimental groups were divided according to HP concentration and experimental exposure time. Forty-two incisors were subjected to in-office non-light-activated tooth bleaching using 25% or 35% HP for 15, 30 or 45 minutes. Four non-bleached incisors were used as the control samples.

The 35% HP (Whitegold Office[b]) was commercially available, and the 25% HP was prepared by the same manufacturer.[b] The bleaching procedures were carried out according to the manufacturer's recommendations. Briefly, the syringe A (hydrogen peroxide) was connected to syringe B (activator) The content A was injected into syringe B and then, the content B was injected into syringe A, and this movement was repeated 20 times to mix A and B contents. The syringes were disconnected, and the mixture was applied on the dental surface. At the end of the bleaching period, the bleaching agent was removed with copious air/water spray, and the teeth were gently dried using sterile gauze.

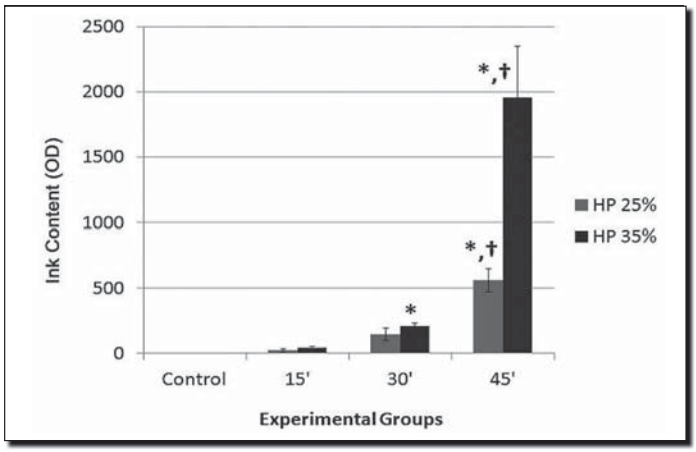

Fig. 1. A graphic representation of the ink content in pixels (± standard error of the mean) of rat incisors made transparent in different experimental groups.
* indicates significant differences from the control group.
† indicates significant difference at 15 minutes

The control and experimental animals were then submitted to an intravascular injection of India ink as previously described by Costa et al[12] with modifications. Briefly, 0.3 mL of India ink was diluted in 0.9% sodium chloride (1:10) and was injected into the lateral caudal vein of the rats. After sufficient time for the complete excretion of the ink from the vascular system (1 hour), the animals were sacrificed by anesthetic overdose, and the jaw and maxilla were removed. Six samples per group were submitted to multiple procedures resulting in the dentin becoming transparent (diaphanization). The samples were immersed in a 2.5% sodium hypochlorite solution for 7 days, washed in running water for 24 hours, and then immersed in a 5% chloride acid solution (that was changed daily) for 5 days. The samples were then washed in running water for 24 hours and incubated in a series of increasingly concentrated ethanol solutions (70%, 80% and 96%; 24 hours per solution). Finally, the samples were immersed in methyl salicylate to render the demineralization dentin transparent.[12] The optical densities (OD) of the crowns were measured using a stereomicroscope at ×40 magnification. The OD (in pixels) of each sample was measured with an imaging program (Adobe Photoshop CS5[c]) and recorded.

For the histological illustration, one sample from each group was immersed in a 10% buffered formalin solution for 24 hours. After 7 days of decalcification in 20% formic acid, the specimens were subjected to routine processing. The paraffin-embedded specimens were sectioned at 5 μm and stained with hematoxylin and eosin.

Each data point represents the mean ± SEM (standard error of the mean) of the optical densities of each group. The data were compared using the Kruskal Wallis test complemented by Dunn's test. The level of significance was 5% ($P \leq 0.05$).

Results

The amount of ink taken up by each experimental group is graphically illustrated in Fig. 1. The OD values of teeth exposed to 35% HP condition for 30 minutes of treatment and of both HP concentrations (25% and 35%) with 45 minutes of treatment were higher than those of the control group ($P < 0.05$). Furthermore, the OD values of both HP concentrations with 45 minutes of treatment suggested higher dye content than after only 15 minutes of treatment.

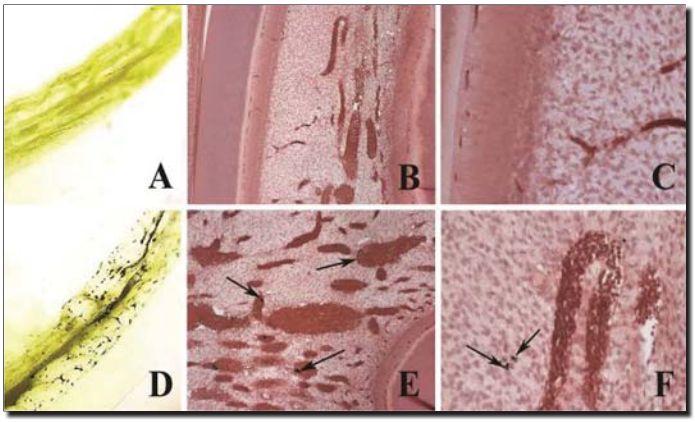

Fig. 2. A representation of the results of dental bleaching. **A.** The control group after diaphanization (at ×40 the original magnification). **B-C.** Histological images of the control group (at ×100 and ×200 the original magnification). **D.** Diaphanization of the 35% HP treated for 45 minutes (at ×40 the original magnification). (**E-F**.) Histological images of the 35% HP treated for 45 minutes (at ×100 and ×200 the original magnification). Note the staining of the dental pulp in the images of the 35% HP-treated tissues and the lack of such staining in the control group.

Figure 2 illustrates the histological features of the samples found to be without (G7) (Fig. 2A-C) and with (Fig. 2D-F) significant ink content. The ink was detected in the vascular walls and connective tissues of the pulpal histological specimens.

Discussion

The possible adverse effects of dental bleaching remain subject to discussion,[14] and the cause of bleaching-related sensitivity is not well understood. It is clear that the bleaching agents are cytotoxic[6,7,15,16] and that photoactivation increases the level of neuropeptides that stimulate pain and inflammatory responses.[13] However, robust evaluations of the biological effects of non-light-activated bleaching agents remain scarce. Furthermore, during bleaching procedures, different HP concentrations and different exposure times are used, and these differences may produce significantly different pulpal responses. Thus, the present study analyzed the inflammatory response of the pulp by measuring the increase in pulpal vascular permeability. The highest increases in vascular permeability were observed in the samples that were bleached using the 25% HP for 45 minutes and the 35% HP for either 30 or 45 minutes.

In vitro studies assessing the safety of bleaching systems have suggested these systems may cause adverse pulpal reactions.[6,7,15-17] However, the absence of host vascular systems and active immune systems in in vitro studies may make these studies less reliable. Thus, in vivo studies are far more appropriate to accurately assess the effects of dental bleaching on the dental pulp. Previous studies have performed in vivo experiments;[9,10,12,13] however, some analyses were performed subjectively through the use of histological sections evaluated with scores.[9,10] Because only isolated sections rather than entire tissues are analyzed in this way, biases can be introduced into the data.

The methods used in this study were previously applied by Costa et al[12] and are based on the principles of pathophysiological inflammation. The capacity of a given substance to induce an inflammatory response can be assessed by measuring the increase of vascular permeability produced by the substance. Our study evaluated vascular permeability using an objective analysis system that measured the quantity of India

ink that leaked from the vascular system that became fixed inside the dental pulp. The dye used (India ink) has a large enough molecular structure that prohibits it from crossing the vascular wall except in conditions of increased vascular permeability; India ink is therefore an appropriate tool for measuring acute inflammatory responses. This method allows for a complete, tridimensional measurement of the dental pulp ink content.

The bleaching agents used produced an acute inflammatory process in the dental pulp, and in all experimental groups produced some degree of ink content. OD analyses confirmed that the quantity of ink leaked from the blood vessels depended on the experimental group. Moreover, the histological images illustrated the location of the ink in the vascular walls and in the interstices of the dental pulp.

Our results show that the duration of dental bleaching is an important factor in causing increased vascular permeability because regardless of the HP concentration used, the amount of ink in the pulp of teeth bleached for 45 minutes was significantly higher than that in teeth bleached for 15 minutes and that in non-bleached control teeth. These results can explain the dentin hypersensitivity after teeth bleaching found by Martin et al[18] in a randomized clinical trial using one application for 45 minutes of HP at 35%. Corroborating our finding that 15 minutes of bleaching does not induce pulpal inflammation, Caviedes-Bucheli et al[13] demonstrated that the production of substance P (released in inflammatory process) in teeth bleached for 15 minutes was similar to that in teeth that were not bleached.

If these pulp specimens were not affected by light, which is capable of inducing inflammatory responses in the pulp,[12,13] then this finding could be explained by the increased exposure of the teeth to substances released by the bleaching agents. In fact, HP is known to have a cytotoxic effect and might therefore be responsible for the inflammatory response observed in the dental pulp. The assay of Costa et al[10] also supports this idea; they have reported that the pulp of the human incisor in vivo is more damaged than that of pre-molars subjected to the same bleaching protocol. According to those authors, this difference occurs because the dentin thickness of incisors is less than that of premolars, allowing a greater amount of the substances released by the bleaching agents to reach the pulp and cause damage.

When bleaching was performed with the 35% HP solution for 30 minutes, an increase in the vascular permeability of the study animals was observed. However, Kina et al[9] did not observe signs of inflammation in the pulp of human teeth bleached with 38% HP for 30 minutes. Although these results seem contradictory, these findings should not be compared because Kina et al[9] analyzed the pulp from 2-15 days after the bleaching procedure; it is therefore not possible to determine whether the pulp in their study also showed an increase in vascular permeability immediately after the procedure.

Rat incisors have enamel on their labial surface but not on their lingual surface. Dentin thickness in rat incisors is only 50 μm thick. Thus, attempts to extrapolate these results directly to humans are not possible. However, these results indicate that in clinical conditions where dentin is very thin, topical treatment with HP may damage pulpal vessels.

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b. Dentsply, Petrópolis, Brazil.
c. Adobe, San Jose, USA.

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