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ORIGINAL ARTICLE

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Edemogenic test and hydrogen peroxide degradation rate of bleaching gels with different desensitizing agents

Teste edemogênico e taxa de degradação do peróxido de hidrogênio de géis clareadores com diferentes agentes dessensibilizantes

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ABSTRACT

Objective: The at-home bleaching technique leads to the intimate contact of the bleaching gel with gingival tissues, so this study evaluated the immediate inflammatory response, through the edemogenic test, induced by at-home bleaching gels of 10% carbamide peroxide with different desensitizing agents, the quantification of hydrogen peroxide released and bleaching gels pH. **Material and Methods:** Forty-eight rats were divided into groups (n=12): CTRL-control group, WP-Whiteness Perfect 10% (FGM Produtos Odontológicos, Joinville, SC, Brazil), OPA-Opalescence 10% (Ultradent Products Inc., South Jordan, IT, USA), and PB-Power Bleaching (BM4, Palhoça, SC, Brazil). For the edemogenic test, all rats received an intravenous injection of Evan's Blue; after 30 min, 0.2 mL of each bleaching gels was injected into the subcutaneous tissue of the rats, and the results of the vascular permeability were assessed after 3 and 6h. The amount of HP released and pH of each product was also determined. Data were submitted to statistical test ($p<0.05$). **Results:** At 3h, the PB showed higher vascular permeability than the other groups. At 6h, the PB produced similar vascular permeability than WHI, and higher than OPA and CTRL groups. The OPA group had a higher vascular permeability at 6h compared to 3h; there is no difference in other groups. The PB group had higher HP concentrations than the other groups. **Conclusion:** In general, the PB caused a more considerable amount of inflammatory edema and higher amount of HP released. This results suggesting that these bleaching gels cause greater aggression in soft gingival tissues that eventually ends up in contact with bleaching products.

KEYWORDS

Tooth bleaching; Carbamide peroxide; Hydrogen peroxide; Capillary permeability.

RESUMO

Objetivo: A técnica de clareamento domiciliar leva ao contato íntimo do gel clareador com tecidos gengivais, assim, este estudo avaliou a resposta inflamatória imediata, através do teste edemogênico, induzido por gel de clareamento caseiro à base de peróxido de carbamida a 10% com diferentes agentes dessensibilizantes, a quantificação de peróxido de hidrogênio liberado e o pH dos géis branqueadores. Material e Métodos: Quarenta e oito ratos foram divididos em 4 grupos (n = 12): grupo-controle CTRL, WP-Whiteness Perfect 10% (FGM Produtos Odontológicos, Joinville, SC, Brasil), OPA-Opalescence 10% (Ultradent Products Inc., South Jordan, IT, EUA) e PB-Power Bleaching (BM4, Palhoça, SC, Brasil). Para o teste edemogênico, todos os ratos receberam uma injeção intravenosa de Evan's Blue; após 30 min, 0,2 mL de cada gel clareador foi injetado no tecido subcutâneo dos ratos, e os resultados da permeabilidade vascular foram avaliados após 3 e 6 horas. A quantidade de HP liberada e o pH de cada produto também foram determinados. Os dados foram submetidos ao teste estatístico ($P <0,05$). Resultados: Às 3h, o PB apresentou maior permeabilidade vascular que os demais grupos. Às 6h, o PB produziu permeabilidade vascular semelhante ao WHI e maior que os grupos OPA e CTRL. O grupo OPA apresentou maior permeabilidade vascular às 6h em relação às 3h; Não existe essa diferença em outros grupos. O grupo PB apresentou maiores concentrações de HP que os demais grupos. Conclusão: Em geral, o PB causou maior quantidade de edema inflamatório e maior quantidade de HP liberado. Estes resultados sugerem que estes géis branqueadores causam maior agressividade nos tecidos gengivais moles que eventualmente acabam em contato com produtos de branqueamento.

PALAVRAS-CHAVE

Clareamento dental; Peróxido de carbamida; Peróxido de hidrogênio; Permeabilidade capilar.

INTRODUCTION

The dental bleaching has been highlighted in esthetic dentistry by delivering impactful results, improving the self-esteem of the patient and their social life [1]. As a proposal for a bleaching treatment, Haywood & Heymann, in 1989 [2], applied bleaching products of the carbamide peroxide (CP) in acetate trays. This treatment was recognized for being technically simple, biologically safe, and aesthetically effective when truly indicated and performed under the supervision of the professional [2,3]..

The CP, when in contact with dental hard tissue, dissociates into urea and hydrogen peroxide (HP) [4], which is the active component responsible for the breakdown of chromogenic agents of the tooth structure [5]. With its low molecular weight, HP penetrates easily through the dental structures, rapidly reaching the pulp tissue [6,7]. It may trigger an inflammatory process [8-10], resulting in different levels of tooth sensitivity [11-13].

To reduce the sensitivity caused by bleaching procedure, some authors reported the use of desensitizing agents in bleaching protocol [12,14-16]. Studies have shown promising results when using potassium nitrate (NP) before or after the HP [12,15,16]. It is believed that this desensitizing agent, upon contact with the dentin–pulp complex, modulates the activity of the sodium (Na+) and potassium (K+) channels present on the cell membrane, thereby affecting the transmission of nerve impulses and decreasing tooth sensitivity [17].

Another desensitizing agent recently incorporated into bleaching protocol is potassium oxalate (PO). This compound was introduced to Dentistry in the 1970s with the intent of treating dentinal hypersensitivity, inducing precipitation in the exposed dentin tubules, reducing the movement of dentinal fluid, and consequently, the pain [17]. Thus, manufacturers incorporated these desensitizing agents into the bleaching gels to reduce the sensitivity during the bleaching technique, making this a safer procedure.

However, the bleaching gel may stay in direct contact with gingival tissues, and the effects of these desensitizers in the soft tissue have not yet been studied. The contact with gingival tissues occurs mainly in the at-home dental bleaching in which the bleaching gel deposited inside the tray may overflow when the tray is placed intra-orally, remaining in contact with the oral soft tissues and mucous membrane of the gastrointestinal tract [18,19].

The most used methods to evaluate the biocompatibility of bleaching gels are *in vitro* studies with cell culture [7] and *in vivo* tests on animals teeth [10] or humans [11]. However, the edemogenic test allows to quantify the level of edema, that shows the immediate response of soft tissues to materials, as the production of edema is proportional to the toxic action of the product [20]. Vascular permeability is a common pathogenic process that is often encountered during the development of inflammation [21], and it is used to assess the irritating potential of dental materials [20,22].

Therefore, this study aimed to evaluate the amount of edema that different bleaching agents cause in the subcutaneous tissue of rats, at different analysis periods. The HP concentration of each product also was evaluated. The null hypotheses were that the different bleaching gels agents would not differ regarding (1) The inflammatory response (regarding edema) in the subcutaneous tissue of rats; (2) The amount of HP released.

MATERIALS AND METHODS

A total of 48 male Wistar rats (*Rattus albinus*), weighing between 200-240 g, were used in this study (n=12). The animals were kept in a temperature-controlled environment at 22°C ± 1°C and under a controlled light cycle (12 h light and 12 h dark), with a solid

diet and water available ad *libitum*. The experimental protocol was approved by the Ethics Committee on the Use of Animals of the Araçatuba Dental School, UNESP—Univ Estadual Paulista (CEUA-n°2014-00787) and the study was conducted in accordance with the ARRIVE guidelines [23].

Experimental design

This article has two studies:

a) In vivo experimental analysis

Factors of analysis

- Bleaching gels in 4 levels (1- Controle, 2- WhitenessPerfect 10%, 3- Opalescence, 4- PowerBleaching)

- Time in 2 levels (3 e 6 hours)

- Response variable – Quantification of edema

b) In vitro experimental analysis:

- Bleaching gels in 4 levels (1- Controle, 2- WhitenessPerfect 10%, 3- Opalescence, 4- PowerBleaching)

- Response variable – The PH degradation rate

Table 1 - Division of groups according to the product evaluated

Group (n=12)	Bleaching active ingredient	Product tested	Desensitizing agent	pH
CTRL	-	Placebo gel	-	7.00
WH	10% Carbamide peroxide	Whiteness perfect*	Potassium nitrate and sodium fluoride	5.94
OPA	10% Carbamide peroxide	Opalescence PF**	Potassium nitrate and sodium fluoride	6.76
PB	10% Carbamide peroxide	Power Bleaching***	3% potassium oxalate	5.64

* FGM Produtos Odontológicos, Joinville, SC, Brazil
**Ultradent Products Inc., South Jordan, IT, USA
***BM4 Materiais Odontológicos e Instrumentos Ltda, Palhoça, SC, Brazil

Edemogenic test to quantify the level of edema

The rats were anesthetized by intramuscular injections of ketamine (80 mg/kg, Ketamina Agener 10%, União Química Farmacêutica Nacional S/A – Embu-Guaçu, SP, Brasil) and xylazine (10 mg/kg, Xilazin, Syntec do Brazil LTDA – Cotia, SP, Brasil). Then, the animals received an intravenous injection of 1% Evan's blue dye (Difco Laboratories, Livonia, MI, USA) diluted in distilled water, at a dosage of 0.2 mL of solution per 100 g of body weight, using a 1 mL insulin syringe, through the penile vein of the animal [20].

After 30 minutes, 0.2 mL of the bleaching gels was injected with insulin syringe subcutaneously into the dorsum region of each rat, near the tail and using the median line as a reference [20], forming the described groups in Table 1.

After 3 and 6 hours, the animals were killed by an overdose of the anesthetic solution (240mg/Kg, Thiopentax; Cristália Produtos Químicos Farmacêuticos LTDA, Itapira, SP, Brazil), followed by manual trichotomy of the dorsal region to reveal the area of edema, and a 23-mm diameter tissue fragment containing a blue halo in the center was removed [20]. These samples were fixed in 4 mL of formamide and stored at 37ºC for 72 h. Then, the samples were filtered, and the solutions were spectrophotometrically analyzed at 630 nm, which is the maximum absorption peak of the dye. Thus, the intensity of the inflammatory edema caused by subcutaneous injection by bleaching gel was determined.

Quantification of hydrogen peroxide released

For quantification of the HP released, five syringes of each bleaching gel were used. The method used is based on titration using oxidation–reduction reaction with potassium permanganate, which provides the amount of HP released by each bleaching gel [23]. The reaction is according to the following formula: $2KMnO_4 + 5H_2O_2 + 4H_2SO_4 = 2KHSO_4 + 2MnSO_4 + 5O_2\_ + 8H_2O$.

The potassium permanganate solution (solution 1) was prepared by mixing 0.2 g of sodium oxalate, 250 mL of distilled water, and 15 mL of sulfuric acid at 80ºC for 30 min [24]. The solution was kept in an amber glass and protected from light for 24 h.

Two grams of each bleaching gels were weighed on an analytical balance and diluted in 10 mL of distilled water (solution 2) to determine the amount of HP in a sample. Then, solution 1 was added to solution 2 at a rate of 0.1 mL/sec, until a violet color was observed. This color change of the solution indicated the equivalence point, i.e., the point at which all HP was consumed. The volume of solution 1 required to change the color of the final solution was applied to the following formula: $C = V \times Cf \times 1.701 \times 100m$, where

C = concentration of hydrogen peroxide (w/w);

V = volume of solution 1 in milliliters added during titration;

Cf = correction factor for the 0.1 N potassium permanganate solution;

m = mass of the sample of the bleaching product in milligrams.

Statistical analysis

Data were tabulated, and the normality and homogeneity of variance assumptions were verified. The data obtained in the edemogenic test were tabulated and submitted to two-way Analysis of Variance, followed by Tukey's test and the data obtained in the hydrogen peroxide concentration were submitted to one-way Analysis of Variance, followed by Tukey's test, both at a significance level of 5%.

RESULTS

Edemogenic Test

The data of edemogenic test can be observed in Table 2. The CTRL group was significantly different to the bleaching gels groups at 3 or 6 h. Regarding the bleaching gel groups, at 3 h, the PB presented higher edema than WH and PB ($p \leq 0,001$), rejecting the first null hypothesis. At 6 h, this difference was found when compared to OPA group; there was no significant difference between the WH and OPA groups in this period. The OPA showed a significant increase of the edema at 6 h ($p=0,029$); there was no difference of the edema in 3 or 6 h from the other groups.

Table 2 - Results of the edemogenic test of bleaching gels

		CTRL	WH	OPA	PB
Edemogenic test*	3 h	0.002 (± 0.001) Ad	1.440 (± 0.112) Ab	1.198 (± 0.200) Bc	1.778 (± 0.118) Aa
	6 h	0.002 (± 0.000) Ac	1.640 (± 0.219) Aab	1.488 (± 0.197) Ab	1.870 (± 0.197) Aa

* Different capital letters indicate significant differences between lines; different lower case letters indicate significant differences between columns ($P<0.05$)

Hydrogen peroxide concentration

The data from the hydrogen peroxide concentration is displayed in Figure 1. When the amount of peroxide released by the bleaching agents was quantified, a statistically significant difference between groups was noted, rejecting the second null hypothesis ($p<0.001$). The PB group had a higher hydrogen peroxide concentration compared to other groups.

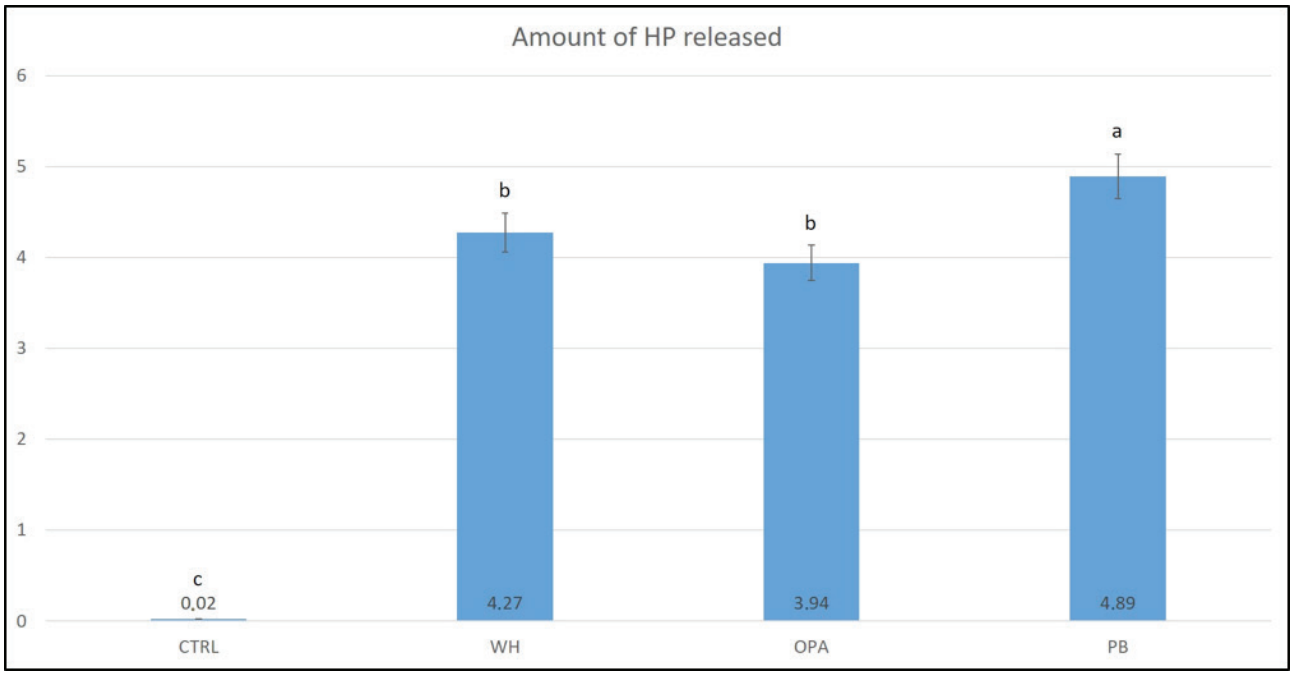

Figure 1 - Results of the quantification of released hydrogen peroxide and pH analysis of bleaching gels (different letters indicate significant differences between columns).

DISCUSSION

This study evaluated the biological response from bleaching gels and showed that bleaching gels with different desensitizing agents in its composition presented different vascular permeability in the subcutaneous tissue of rats. Furthermore, bleaching gels with the same concentration of CP promoted the different amount of HP released.

Most commercially available at-home whitening products produce levels of hydrogen peroxide ranging from 3.5% to 7.5%, based on carbamide peroxide at concentrations of 10% to 22% [12,24]. Due to the relatively low concentrations of hydrogen peroxide, the at-home technique has been considered safe and effective, when monitored by professionals [25].

However, some side-effects can still be observed, particularly when the gel comes into contact with the soft tissue [3,26]. In vivo studies evaluating the degree of gingival inflammation after exposure to the bleaching gels used at-home show that between 25% and 40% of patients experienced some level of gum irritation during the dental bleaching, and this fact, along with tooth sensitivity, results in more complaints by patients during treatment [27]. In another clinical study, Carlos et al. evaluated home bleaching agents using different types of trays. They observed that all patients submitted to bleaching treatment, regardless of the type of tray, presented significant gingival irritation caused by agents bleaching agents [28]. In an in vitro study, Furukawa et al. evaluated the genotoxic effect of hydrogen peroxide on human gingival fibroblasts. In this study, they used hydrogen peroxide at low concentrations and observed that there is a stimulation of the proinflammatory tumor necrosis factor (TNF) cascade in cytokines in gingival fibroblasts due to contact with the bleaching agents [29].

Based on these clinical observations, this study used methods previously used in endodontics [30] and quantified the vascular permeability caused by direct contact of various bleaching gels to the connective tissue of rats. This analysis is based on determining the optical density of a solution containing the dye that binds to albumin, a protein that is present in large amounts in inflamed areas [31]. Therefore, the higher the optical density of this solution, the greater the inflammation at the site. Thus, the results of this study indicate, indirectly, the

inflammatory potential of at-home bleaching gels when they come into contact with mucous membranes. Furthermore, the analysis of edema generated by 3 and 6 h of contact with the products relates, respectively, to the approximate time typically recommended in the daytime and nighttime use options of the tray.

This study showed that all bleaching gels produced vast edema when compared to the control group. Within 3 h, the gel Power Bleaching® 10% with potassium oxalate resulted in markedly more edema than other groups. It may be related to the high toxicity of potassium oxalate to the skin and mucous membranes [32]. It is rather worrisome since at-home gels often come into contact with the soft tissues, which can result in tissue irritation. Also, differences in formulations that were not analyzed in this study, as well as recent changes in pH during the presence of the product in tissues may have contributed to the intense and immediate response produced.

The effectiveness of potassium oxalate was initially proven when the product was applied topically to the exposed dentine structure or teeth with hypersensitivity [33]. However, these clinical conditions represent major contraindications for dental bleaching, as they may encourage excessive penetration of peroxides into the tooth pulp complex [34]. Thus, the possible benefits of using an at-home gel containing potassium oxalate should be studied carefully, as they have direct contact only with the tooth enamel, showing results inferior to other at-home bleaching gels.

Nevertheless, the source of discomfort in hypersensitive teeth is a very distinct sensitivity caused by the dental bleaching. Even considering the superior results reported by Bernardon et al. [35], it is difficult to explain how potassium oxalate could help to relieve the sensitivity caused by penetration of peroxide into the pulp tissue. It is known that the painful sensation caused by bleaching treatment is primarily related to an inflammatory process caused by the action of reactive oxygen species in the pulp cells [7-10,36,37], in addition to the occupation of specific nociceptors in this tissue [38]. On the other hand, the tooth hypersensitivity is primarily related to the dynamics of dentinal fluids [39].

Regarding the amount of hydrogen peroxide in each bleaching gel, Goldstein et al. [40] reported that 10% carbamide peroxide-based products, when in contact with water or saliva, release approximately 3.5% hydrogen peroxide. Nevertheless, all gels tested in the present study released higher levels of hydrogen peroxide, notable in the G3 group (containing PO), that produced 4.98% hydrogen peroxide. This concentration results in a faster color change during treatment than obtained with the other products evaluated, but can also cause a more intense tissue response.

Despite the limitations of the experimental model, this study highlights the need for further studies with bleaching gels containing potassium oxalate, since these products may promote a pronounced tissue response, suggesting that these bleaching gels cause greater aggression in soft gingival tissues that eventually ends up in contact with bleaching products. Furthermore, different bleaching gels labeled as having the same concentration of carbamide peroxide can release different amounts of hydrogen peroxide, which should alert clinicians to the care they should take to indicate dental bleaching, regardless the concentration of the gel used.

CONCLUSION

In conclusion, the bleaching gels that promoted the higher amount of HP release caused higher vascular permeability. Also, the bleaching gels with potassium oxalate are related to a more intense tissue response.

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