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Administration of Ascorbic Acid to Prevent Bleaching-induced Tooth Sensitivity: A Randomized Triple-blind Clinical Trial

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Clinical Relevance

The use of ascorbic acid during in-office bleaching does not reduce the incidence of bleaching-induced tooth sensitivity.

SUMMARY

This study evaluated the effect of ascorbic acid, 500 mg every eight hours, on bleaching-induced tooth sensitivity. A triple-blind, parallel design, and placebo-controlled randomized clinical trial was conducted on 39 adults. The pills (placebo or ascorbic acid) were administered three times per day for 48 hours; the first dose was given one hour prior to each bleaching session. Two bleaching sessions with 35% hydrogen peroxide gel were performed with a one-week interval. Tooth sensitivity was recorded up to 48 hours after bleaching. The color evaluation was performed before and 30 days after bleaching. The absolute risk and intensity of tooth sensitivity were evaluated by Fisher exact and Mann-Whitney U-tests, respectively. Color changes were evaluated by unpaired *t*-test (α=0.05). There were no significant differences in the absolute risk and intensity of tooth sensitivity and color change between the groups. Both groups showed a similar risk of tooth sensitivity ($p>0.05$). The perioperative use of an antioxidant, such as ascorbic acid (500 mg, three times daily) perorally, was not able to prevent bleaching-induced tooth sensitivity or reduce its intensity.

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INTRODUCTION

The desire for whiter teeth has made tooth bleaching one of the most sought-after cosmetic procedures in

dentistry.[1] Various agents can be used to whiten teeth, such as hydrogen peroxide (HP), carbamide peroxide, and sodium perborate. These materials can penetrate the enamel and dentin structures, releasing reactive oxygen radicals that oxidize chromogens.[2] Available bleaching modalities include dentist-supervised in-office bleaching, dentist-prescribed home-applied bleaching, and over-the-counter consumer-available systems.[3]

The in-office procedure using 35% HP has a long history of tooth sensitivity (TS) and gingival irritation.[3-6] Prevalence levels of TS have been reported to vary from 55% to 90%.[4-8] This sensitivity seems to result from the easy passage of the HP through the enamel and dentin to the pulp,[9] causing pulp damage and inflammation.[10]

This cell damage is probably the result of oxidative stress produced by HP and its by-products on cells. HP is a potential source for hydroxyl radicals, one of the most dangerous radicals. It was reported that HP and its by-products, superoxide anions and hydroxyl radicals, are injurious to cells via oxidative stress,[11] and they were able to cause cytotoxicity, apoptosis, and genotoxicity in mouse P388 cells.[12] Along with cell damage, cell-derived factors, such as ATP[13] and prostaglandins, are released, exciting and sensitizing pulp nociceptors,[14] leading to the transmission of the pain stimuli.

In an attempt to reduce the damage produced by HP on the pulp, some authors[12,15,16] have investigated the role of antioxidants. It was reported that the use of a flavonoid, which is a naturally occurring antioxidant (naringin), on dentin before HP application reduced the H_2O_2-induced cytotoxicity, apoptosis, and genotoxicity[12] under *in vitro* conditions. This antioxidant was also shown to suppress the DNA damage induced by HP. Other authors reported that the application of 10% sodium ascorbate on dentin discs, before the application of a carbamide peroxide gel, reduced the cytotoxic effects of these products on cells,[15] and these effects were shown to be directly related to the HP concentration and the period the antioxidant was left on the tooth surface.[16]

The results of the aforementioned studies indicate that the presence of a natural product with antioxidant and anti-apoptotic properties on pulp could reduce the damage produced by the in-office bleaching products, and this could be clinically translated to a reduction of the risk of TS and its intensity. However, the only available method to deliver an antioxidant to the pulp tissue is through either a peroral route or intravenously. Therefore, this study attempted to investigate if the perioperative use of an antioxidant perorally during in-office bleaching could reduce the oxidative stress produced by HP on pulp cells, and thus, reduce the risk and intensity of bleaching-induced TS. Three null hypotheses were tested: 1) the perioperative use of ascorbic acid, starting one hour before the in-office bleaching session, will not reduce the absolute risk of TS; 2) the use of this antioxidant drug will not reduce the intensity of TS; and 3) the use of this antioxidant will not affect the degree of tooth whitening.

MATERIALS AND METHODS

This clinical investigation was approved (protocol 17836/2010) by the scientific review committee and the committee for the protection of human subjects of the State University of Ponta Grossa (Ponta Grossa, PR, Brazil). The experimental design followed the Consolidated Standards of Reporting Trials statement.[17] Thirty-nine volunteers from the city of Guarapuava, PR, Brazil were selected for this study in the clinic of the Brazilian Association of Dentistry in Guarapuava from May 2011 to June 2012. Two weeks before the bleaching procedures, all of the volunteers received a dental prophylaxis with pumice and water in a rubber cup and signed an informed consent form.

Inclusion and Exclusion Criteria

Participants included in this randomized, triple-blind, placebo-controlled with a parallel-group clinical trial were at least 18 years old and had good general and oral health. Participants were recruited by means of radio and television advertisement. The participants were required to have at least six maxillary and mandibular anterior teeth that were caries-free and without restorations on the labial surfaces. Selected participants had central incisors that were shade C2 or darker, as judged by comparison with a value-oriented shade guide (Vita Lumin, Vita Zahnfabrik, Bad Säckingen, Germany).

Participants who had previously undergone tooth-whitening procedures or had preexisting anterior restorations or internal tooth discoloration (tetracycline stains, fluorosis, pulpless teeth) were not included in the study. Pregnant and lactating women and participants taking any medicine were not included in the study. Additionally, participants with bruxism habits or any pathology that could cause TS (eg, recession, dentin exposure) were excluded. This was done to minimize confounding experimental variables or side effects from bleaching. Participants who reported a history or presented

health problems in the stomach, heart, kidney, or liver, or participants using any continuous drug with anti-inflammatory and antioxidant action were also excluded from the study.

Study Intervention

Participants were randomly divided into the placebo (n=19 participants) and ascorbic acid groups (n=20 participants). The randomization process was performed using computer-generated tables by a third person (statistician), not involved in the research protocol. Details of the allocated groups were recorded on cards contained in sequentially numbered, opaque, sealed envelopes. Once the participant was eligible for the procedure and completed all baseline assessments, the allocation assignment was revealed when the research assistant opened this envelope. Neither the participant nor the operator (E.A.P.) knew the group allocation, being both blinded to the protocol.

The participants from the placebo group received a placebo pill (Talco pharma S M-200 Henrifarma, São Paulo, SP, Brazil), and participants from the experimental group received a 500 mg dose of ascorbic acid (pill, vitamin C, Citroplex, Lab Neo Química, Anápolis, GO, Brazil). All of the participants were watched to ensure that they took the drugs or placebo one hour prior to treatment. The other doses of placebo or ascorbic acid were administered every eight hours after the first dose over a period of 48 hours. Participants were reminded by a research assistant via telephone to take their doses of ascorbic acid/placebo. This procedure was done to increase adherence to the protocol.

The medicine was administered for 48 hours because, although bleaching-induced tooth sensitivity complaints usually cease within the first 24 hours, some patients have reported pain up to 48 hours after treatment.[18] We have selected the minimal dosage of ascorbic acid available on the Brazilian market. The medicine was administered every eight hours because the concentration of ascorbic acid is almost minimal eight hours after ingestion.[19]

The gingival tissue of the teeth to be bleached was isolated from the bleaching agent using a light-cured resin dam (Top Dam, FGM, Joinville, SC, Brazil). The 35% HP gel (Whiteness HP Maxx, FGM) was used in three 15-minute applications for both groups following the manufacturer's directions. The in-office bleaching agent was refreshed every 15 minutes during the 45-minute application period. Two bleaching sessions, with a one-week interval, were performed on each patient (E.A.P.). All participants were instructed to brush their teeth at least three times a day using a fluoridated toothpaste (Sorriso Fresh, Colgate-Palmolive, São Paulo, SP, Brazil) provided by the investigators.

Shade Evaluation

Shade evaluation was recorded before and 30 days after the bleaching treatment using two methods: subjective evaluation using a value-oriented shade guide (Vita Lumin, Vita Zahnfabrik) and an objective evaluation using the Easyshade spectrophotometer (Vident, Brea, CA, USA).

For the subjective examination, the shade guide's 16 tabs were arranged from highest (B1) to lowest (C4) value, making the minimum qualifying shade C2 as number 7 (seventh tab on the value-ordered arrangement). Although this scale is not linear in the truest sense, we treated the changes as representing a continuous and approximately linear ranking for the purpose of analysis. The measurement area for shade matching was the middle third of the facial surface of the anterior central incisor. This measurement was done at baseline and 30 days after bleaching, allowing for the calculation of means and standard deviations of delta shade guide units (ΔSGU) of each group.

For calibration purposes, five participants whom we did not include in the sample participated in the training phase of this study. The two examiners (A.R. and A.D.L.), blinded to the allocation assignment, scheduled these participants for bleaching and evaluated their teeth against the shade guide at baseline and 30 days after the procedure. The two examiners were required to have an agreement of at least 85% (kappa statistic) before beginning the study evaluation. During the study, if disagreements arose, the examiners reached a consensus before dismissing the patient.

For the objective evaluation, a preliminary impression of the maxillary arch was made using dense silicone Adsil (Vigodent, Rio de Janeiro, RJ, Brazil). The impression was extended to the upper canine and served as a standard shade measurement guide for the spectrophotometer. A window was created on the labial surface of the molded silicone guide for the central incisor to be evaluated. The window was made using a metallic device with well-formed borders, 3 mm in radius.[5] The measurement was done on all 39 participants using the Vita Easyshade spectrophotometer (Easyshade, Vident) before and

30 days after the bleaching therapy by only one operator (E.A.P.). The shade was determined using the parameters of the Easyshade device where it indicated the following values: L*, (a*), and (b*), in which L* represents the value from 0 (black) to 100 (white) and a* and b* represent the shade, where a* is the measurement along the red-green axis and b* is the measurement along the yellow-blue axis. The shade comparison before and after treatment was given by the differences between the two shades (ΔE), which is calculated using the formula[20-22]: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Tooth Sensitivity Evaluation

The patients recorded their perception of TS during the first and second bleaching sessions using two pain scales. A five-point verbal rating scale [0 = none, 1 = mild, 2 = moderate, 3 = considerable, and 4 = severe][5,6,8] and a visual analog scale[23-26] using a 10-cm horizontal line with words "no pain" at one end and "worst pain" at the opposite end were employed in this study. We asked subjects to record whether they experienced TS during the treatment and up to 48 hours after bleaching.

As two bleaching sessions were performed, the worst scores/numerical values obtained in both bleaching sessions were considered for statistical purposes. The values were arranged into two categories: absolute risk of TS, which was the presence of TS at any assessment point, and intensity of TS at each assessment point. These values were computed only for the maxillary arch.

Statistical Analysis

For sample size calculation, the primary outcome was the absolute risk of TS. The absolute risk of TS was reported to be approximately 90%[8,27-29] for the bleaching product Whiteness HP Maxx (FGM). In order to be able to detect an absolute difference of 40% between the placebo and the experimental group, a minimum of 17 participants were required with a power of 80% and alpha of 5%.

The data analysis followed the intention-to-treat protocol and involved all participants who were randomly assigned.[17] The statistician was blinded to the study groups. The primary outcome absolute risk of TS was compared by using the Fisher exact test (alpha=5%). The relative risk, as well as the confidence interval for the effect size, was calculated.

The data sets of TS intensity were plotted on histograms and inspected for normal distributions. As the data did not appear to be normally distributed, nonparametric statistical tests were used. For each pain scale, a comparison of the two groups at the three different assessment points was performed using the Mann-Whitney U-test. Comparisons between times within each group were performed using the Friedman tests. In all statistical tests, the significance level was 5%.

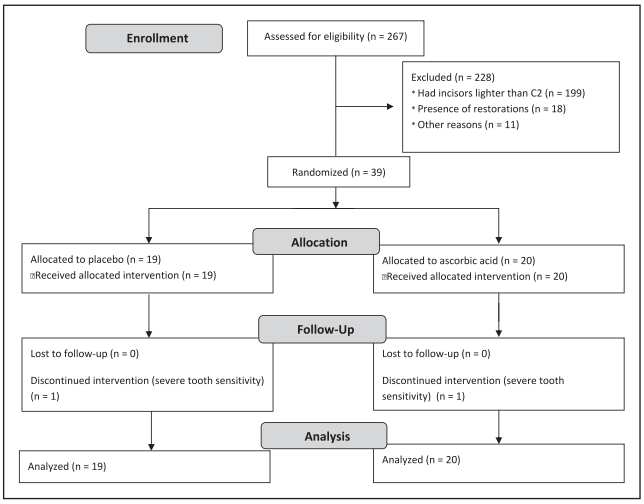

Figure 1. *Flow diagram of the clinical trial, including detailed information on the excluded participants.*

Shade change, another secondary endpoint, was used to assess the efficacy of the bleaching treatment associated with perioperative use of ascorbic acid. The data from ΔSGU and ΔE values of both groups were compared by the Student *t*-test. In all statistical tests, the significance level was set at alpha of 5%.

RESULTS

A total of 267 participants were examined to select 39 participants for the study (Figure 1). The mean ages (years) of the participants in this study were similar between the groups (placebo: 25.3 ± 6.7 years and ascorbic acid: 28.3 ± 9.7 years, *t*-test, $p=0.832$). The baseline colors (SGU) were also similar between the groups (placebo: 9.5 ± 1.9 and ascorbic acid: 9.9 ± 1.8; *t*-test, $p=0.552$). Of the participants from the placebo and ascorbic acid groups, 53% and 35% were male, respectively. Figure 1 depicts the participant flow diagram in the different phases of the study design.

Tooth Sensitivity

The data from 39 participants were used in this study, following the intention-to-treat analysis. One patient from the ascorbic acid group received an

Table 1: *Comparison of the Number of Patients Who Experienced Tooth Sensitivity (TS) at Least Once During the Bleaching Regimen in Both Groups Along With Absolute and Relative Risks**

Treatment	Number of Participants With TS		Absolute Risk (95% CI)	Relative Risk (95% CI)
	Yes	No		
Placebo	16	3	84.2 (62.4-94.5)	1.05 (0.78-1.41)
Ascorbic acid	16	4	80 (58.4-91.9)	

* Fisher test (p=1.0).

analgesic after the first bleaching session, and one patient from the placebo group received an analgesic after the second bleaching session due to severe TS.

With regard to the absolute risk of TS, no significant difference was observed between groups (Table 1; $p=1.00$). The relative risk, along with the 95% confidence interval, is also evidence that the use of the experimental drug had no effect on the TS reduction.

Most of the TS complaints occurred within the first 24 hours, and only two participants experienced pain after 24 hours. With regard to TS intensity (Table 2), the groups did not differ statistically under the two pain scales used in this study ($p>0.05$).

Significant whitening was observed in both study groups under the subjective and objective evaluation methods ($p<0.001$). A whitening of approximately 5.7 and 5.6 SGU were detected for placebo and ascorbic acid groups, respectively (Table 3). A variation of 8.0 and 7.0 in the ΔE was observed for the placebo and ascorbic acid groups, respectively (Table 3). The results of the subjective (visual shade guide) and the objective evaluation (spectrophotometer) matched the hypothesis of equality between the groups after bleaching ($p \asymp 0.6$ for both methods).

DISCUSSION

HP is a reactive oxygen species (ROS) frequently found within the cells as the result of a series of intracellular reactions that occur specifically in the mitochondria.[30] Whether produced endogenously as a consequence of normal cell functions or derived from external sources, ROS pose a constant threat to living cells because they can cause severe damage to DNA, protein, and lipids. Cells contain a number of antioxidant defenses to minimize fluctuations in ROS, but when ROS generation and/or exposure exceeds the antioxidant capacity of cells, a condition termed oxidative stress occurs.[31] This may be the cause of pulp damage produced by in-office bleaching.[10]

Although HP, by itself, is relatively nonreactive toward DNA, most of the HP-mediated damage is due to the production of the hydroxyl radical, a by-product of the HP degradation. The hydroxyl radical is an extremely reactive oxidant; it can react rapidly with DNA and can cause over 100 different types of DNA modification.[30] Therefore, the increase in the exogenous levels of these highly reactive free radicals in contact with cells, as it occurs during in-office bleaching with HP, may result in cell death and reduction of cell proliferation.[32-35]

As mentioned in the Introduction, several studies have proposed the use of antioxidant agents for treatment and/or prevention of the oxidative stress caused by HP from bleaching,[12,15,16] and promising findings have been reported. Among antioxidants available for oral administration, ascorbic acid, also known as vitamin C, is the most popular, and gram doses are promoted for preventing and treating the common cold, managing stress, and enhancing well-

Table 2: *Comparison of Tooth Sensitivity Intensity Experienced by Patients From the Treatment Groups at Different Assessment Points Using Two Pain Scales**

Time Assessment	0-4†		0-10†	
	Placebo	Ascorbic Acid	Placebo	Ascorbic Acid
Up to 1 hour	2 (0/2) aA	1 (0/2.75) aA	2.2 (0/3.2) aA	1.6 (0/3.3) aA
1 to 24 hours	2 (0/2) aA	1 (0/3) aA	1.6 (0/4.7) aA	2.2 (0/3.2) aA
24 to 48 hours	0 (0/0) aB	0 (0/0) aB	0 (0/0) aB	0 (0/0) aB

** Comparisons are valid only within the same pain scale. At each period, the two treatments were compared with the Mann-Whitney U-test and differences are represented by different lowercase letters. For each treatment, the three periods were compared with the Friedman test ($\alpha=0.05$,), and differences are represented by different uppercase letters. † Medians (first/third quartile) values.*

Table 3: *Means and Standard Deviations of the Change in Shade Guide Units (Vita Classical Shade Guide, [ΔSGU]) and ΔE (Spectrophotometer) Between Baseline and 30 Days After Bleaching for the Two Treatment Groups**

		Placebo	Ascorbic Acid	*p*-Value
Subjective evaluation	ΔSGU	5.7 ± 1.8 A	5.6 ± 2.9 A	0.86
Objective evaluation	ΔE	8.0 ± 3.0 A	7.0 ± 3.6 A	0.28

** Comparisons are only valid within rows. Means indicated by the same uppercase letters indicate statistically similar means (Student t-test, α=0.05).*

being. Ascorbic acid is an electron donor, and because of this, is a potent water-soluble antioxidant in humans.[36] In addition, ascorbic acid has been studied for the treatment of several well-known diseases such as hypertension[37] and cancer,[38] due to its ability to minimize the harmful effect of free radicals, reducing the oxidative stress on cells.

However, contrary to the current authors' expectations, the perioperative use of ascorbic acid did not reduce the bleaching-induced TS, which led us not to reject the first and second null hypotheses. Unfortunately, results from *in vitro* studies cannot necessarily be extrapolated to the clinical situation. It is probable that the amount of antioxidant delivered to cultured cells in the *in vitro* studies[12,15,16] was much higher than the level of antioxidant reached in the extracellular fluid after oral administration of 500 mg of ascorbic acid. By using the peroral route of administration, several factors such as the presence of the immune system, lymphatic drainage, urinary excretion, and morphologic characteristics of the dentin substrate may modulate the amount of ascorbic acid that reaches the plasma and extracellular fluid[19] around pulp cells.

Clinical pharmacokinetic studies have shown that ascorbic acid concentrations in plasma and tissues were tightly controlled under oral administration.[39] At doses lower than 100 mg/day, there is a steep sigmoidal relationship between dose and concentrations. At doses higher than 100 mg/day, plasma concentrations reach a plateau between 70 and 80 μmol/L. At doses greater than 400 mg/day, further increases in plasma concentrations were minimal.[39] Thus, the administration of higher dosages of ascorbic acid than the one given in this study would not be expected to provide additional benefits.

On the other hand, when ascorbic acid is administered intravenously, the limiting absorptive mechanism is bypassed, and high plasma levels are attained.[19] For instance, following the administration of 1.25 g intravenously, a peak plasma level of 1000 μmol/L is reached, even though 100 μmol/L is not exceeded by oral dosing.[40] Therefore, one could speculate that the intravenous administration of ascorbic acid could be an option to increase the concentration level of ascorbic acid within and around pulp cells, although intravenous administration is not suitable for routine use in bleaching procedures.

Another option would be the topical application of ascorbic acid on the enamel surface, as a way to allow fast delivery of the antioxidant to the pulp tissue; however, it is probable that the ascorbic acid would not penetrate the enamel substrate, by diffusion alone, due to its organic composition and high molecular weight (176.09 g/mol). This may be a possibility by using dielectrophoresis to drive drugs directly into site-specific intraoral targets. This technology could transport drugs "directly" into teeth using an alternating current electric field. When this technology becomes clinically feasible, it may reduce the oral systemic route of many drugs, overcoming the disadvantages of peroral route administration, and allowing fast delivery of drugs to the pulp tissue.[41]

With regard to the bleaching outcome, the results of this study indicated that both groups demonstrated equivalent and significant tooth shade enhancement when compared with the baseline (Table 3), and thus the third null hypothesis was not rejected. The comparison of shade change after in-office bleaching with existing literature is difficult, due to the different methods of measurement (shade guides and spectrophotometers) and different units of measurement (eg, CIELab system, shade guide units) employed. However, studies that used 35% HP and reported their results in shade guide units usually observed an overall shade change of 5 to 8 shade guide units after two bleaching sessions,[6,42-45] which is in agreement with the results of the present investigation.

This study was designed to find a high effect size, ie, a difference in 40% in the TS among participants from the experimental and placebo groups. Thus, we can conclude that an effect as large as this was not observed, but we cannot rule out the fact that smaller effect sizes do exist. Conducting the same

experimental design using higher sample sizes should be encouraged to rule out this hypothesis. Additionally, the sample selected is mainly composed of young participants, which also limits the ability to generalize for older adults.

CONCLUSIONS

Within the limitations of the current study, we conclude that the use of ascorbic acid, 500 mg three times daily, does not reduce the absolute risk and intensity of TS. However, this study was designed to detect a high effect size, and thus we cannot entirely rule out the benefits of ascorbic acid on TS.

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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