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ELOISA ANDRADE DE PAULA

EFEITO DE DOIS ANTI-INFLAMATÓRIOS E UM ANTIOXIDANTE NA PREVENÇÃO DA SENSIBILIDADE CAUSADA PELO CLAREAMENTO DENTAL

PONTA GROSSA
2013

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Tese apresentada como pré-requisito para obtenção do título de Doutor na Universidade Estadual de Ponta Grossa, no curso de Doutorado em Odontologia – Área de Concentração em Dentística Restauradora.
Linha de pesquisa: Pesquisa clínica em odontologia.

Orientadora: Prof[a]. Dr[a]. Alessandra Reis
Co–oritendador: Prof. Dr. Alessandro Dourado Loguercio.

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Ponta Grossa, 05 de dezembro de 2013.

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A ***Deus***, pela vida.

A minha família: meus pais, Antônio Carlos de Paula ("in memoriam") e Carmem Lucia Andrade de Paula, fontes inesgotáveis de amor e dedicação, exemplos de luta que me ensinaram a vencer os desafios e a levantar a cada tropeço, em especial, a meu pai que tanto sonhou com a realização deste trabalho, incentivando-me até mesmo nas horas mais difíceis em que ele mais precisou de incentivo; ***meus irmãos, João Daniel Andrade de Paula e Carlos Antônio de Paula ("in memoriam"),*** o primeiro pela sua presença em minha vida, o segundo pelo aprendizado e amadurecimento com a sua ausência.

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RESUMO

Paula, EA. **Efeito de dois anti-inflamatórios e um antioxidante na prevenção da sensibilidade causada pelo clareamento dental.** [Tese - Doutorado em Odontologia Área de Concentração em Dentística Restauradora – Universidade Estadual de Ponta Grossa; 2013].

As técnicas de clareamento dental em dentes vitalizados podem apresentar o inconveniente de causarem sensibilidade dental. Neste estudo foram realizados três experimentos com o objetivo de determinar o efeito de dois anti-inflamatórios e um antioxidante utilizados previamente e durante o procedimento de clareamento dental em consultório na sensibilidade dental e no grau de clareamento alcançado. Estes experimentos foram do tipo estudo clínico, randomizado, paralelo, controlado por placebo e triplo cego. No experimento 1, os participantes receberam placebo ou ibuprofeno 400 mg a cada 8 h. No experimento 2, os participantes receberam placebo ou etoricoxibe 60 mg a cada 24 h. No experimento 3, os participantes receberam placebo ou ácido ascórbico 500 mg a cada 8 h. Em todos os experimentos os medicamentos foram administrados 1 hora antes do início do tratamento clareador por um período total de 48 h. Em cada sessão de clareamento, o gel de peróxido de hidrogênio 35% (Whiteness HP Maxx, FGM) foi aplicado 3 vezes durante 15 minutos cada, de acordo com as instruções do fabricante e repetido depois de uma semana, seguindo o mesmo protocolo. As alterações de cor foram avaliadas antes e 30 dias após o tratamento clareador utilizando o espectrofotômetro Vita Easy Shade e a escala de cor Vita Clássica organizada por ordem de valor. Três escalas destinadas a avaliar a intensidade da dor (escala verbal de 5 pontos, escala numérica de 101 pontos e escala visual analógica) foram utilizadas para determinar o nível de sensibilidade imediatamente até 1 h, 24 h e 48 h após o tratamento de clareamento em consultório. A porcentagem de pacientes que relataram sensibilidade, ao menos uma vez durante o tratamento, e a intensidade da sensibilidade dental foram avaliadas pelo teste exato de Fischer e teste de Mann-Whitney, respectivamente. As alterações de cor dental foram determinadas por medidas repetidas ANOVA 1 critério e teste t. Não foram detectadas diferenças significativas no percentual de pacientes que apresentaram sensibilidade e nem na alteração de cor. Somente o medicamento ibuprofeno 400 mg apresentou redução da intensidade da sensibilidade na primeira hora após o tratamento clareador. Os medicamentos não foram capazes de prevenir e nem mesmo minimizar a sensibilidade dental quando administrados de acordo com a posologia e dose empregadas neste estudo.

Palavras-chave: clareamento dental, ensaio clínico controlado aleatório, sensibilidade da dentina, peróxido de hidrogênio.

ABSTRACT

Paula, EA. **Effect of two anti-inflammatories and one antioxidant to prevent bleaching-induced tooth sensitivity.** [Tese - Doutorado em Odontologia Área de Concentração em Dentística Restauradora – Universidade Estadual de Ponta Grossa; 2013].

The tooth bleaching has a long history of tooth sensitivity (TS). This study aimed to determine the effect of different drugs, administered perioperatively, on tooth sensitivity and the degree of tooth whitening after in-office bleaching. A triple-blind, parallel design, randomized clinical trial were conducted. In the first experiment, the participants received a placebo or 400 mg ibuprofen every 8 h while in the second experiment, they were given a placebo or 60 mg etoricoxib every 24 h. In the third experiment, they received a placebo or 500 mg ascorbic acid every 8 h. In all experiments, the first dose of the drug was administered 1 h before in-office bleaching and taken for a period 48 h. The 35% hydrogen peroxide gel (Whiteness HP Maxx, FGM) was used in three 15-min applications for three groups following the manufacturer's directions. The in-office bleaching agent was refreshed every 15 min. Two bleaching sessions, with a one-week interval, were performed on each patient. The shade evaluation were performed before and 30-days, after bleaching with a Vita Easy Shade spectrophotometer and a Vita Classical's visual shade guide. The tooth sensitivity was recorded on three scales: the five-point verbal rating scale, the 101 points scale, and the visual analogue scale, of and in different time points up to 1 h, 24 h, 48h after bleaching. The % of patients that reported TS at least once during treatment were evaluated by Fisher`s exact and the intensity of tooth sensitivity with Mann-Whitney test. Tooth color changes were evaluated by repeated ANOVA measures and t-test. There were no significant differences in the % of patients with tooth sensitivity and color change between the groups. The lowest tooth sensitivity was observed in the 400 mg ibuprofen group only up to 1 h post bleaching. The perioperative use of ibuprofen, etoricoxib and ascorbic acid per oral route was not able to avoid bleaching-induced tooth sensitivity according to the posology and dose used in this study. However, the 400 mg ibuprofen was able to reduce the intensity of tooth sensitivity up to 1 h post bleaching.

Key words: Tooth bleaching, randomized controlled trial, dentin sensitivity, hydrogen peroxide.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS

AA	-	Ácido Araquidônico
ADA	-	American Dental Association
ANOVA	-	Análise de variância
Cf.	-	Conforme
cm	-	centímetro(s)
COEP	-	Comissão de Ética em Pesquisa Envolvendo Seres Humanos
COX	-	Enzima ciclo-oxigenase
EROS	-	Espécies Reativas de Oxigênio
EVA	-	Escala Visual Analógica
h	-	hora
H_2O_2	-	Peróxido de Hidrogênio
min	-	minuto
mg	-	miligrama(s)
PGs	-	Prostaglandinas
TV	-	Televisão
UCV_0	-	Unidade de Cor da escala Vita inicial
UCV_1	-	Unidade de Cor da escala Vita final
UEPG	-	Universidade Estadual de Ponta Grossa
ΔUCV	-	Variação das Unidades de Cor da escala Vita
ΔE	-	Variação de cor

LISTA DE SÍMBOLOS

=	Igual
±	Mais ou menos
%	Porcentagem
<	Menor
≤	Menor ou igual
>	Maior
≥	Maior ou igual
α	Alfa (nível de significância)
n°	Número
p	Significância estatística
®	Registrado
™	Marca registrada
Δ	Delta

SUMÁRIO

1 INTRODUÇÃO

O sorriso é um dos principais fatores da habilidade de interação e comunicação de uma pessoa, por isso muitos pacientes, além de preocupados com a saúde bucal, estão em busca do sorriso perfeito que se traduz na harmonia entre os dentes, gengiva, lábios e face (Joiner[1] 2004). Joiner[1] (2004), relata que 28% dos pacientes estão insatisfeitos com a aparência dos dentes e 34% estão descontentes com a cor. Existem muitos materiais e métodos de tratamento para melhorar a cor dental, entre eles o tratamento clareador, que utiliza produtos oxidantes como o peróxido de hidrogênio que por ser um tratamento conservador, de resultados rápidos e de baixo custo, tornou-se popular e é amplamente empregado (Joiner[2] 2006).

Apesar de seguro (Giniger, MacDonald[3] 2005) esse procedimento induz à sensibilidade dental (Haywood[4] 2005) que tem sua etiologia provavelmente relacionada à agressão do tecido pulpar (Markovwitz[5] 2009) em virtude do contato direto desse tecido com o peróxido de hidrogênio. Ao atravessar o esmalte e a dentina o peróxido pode causar pulpite (Cooper, Bokmeyer, Bowles[6] 1992), que, apesar de reversível (Cooper, Bokmeyer, Bowles[6] 1992, Cohen[7]1979, Robertson et al.[8] 1980) chega a atingir graus de sensibilidade que leva alguns pacientes a abandonarem a terapia clareadora (Schulte et al.[9] 1994).

Existem duas formas viáveis de tratamento clareador: clareamento caseiro e clareamento realizado em consultório (Leonard et al.[10] 2007). O clareamento caseiro é utilizado com frequência para clarear dentes vitais, porém, alguns pacientes não se adaptam ao uso da moldeira e nem pretendem esperar de duas a três semanas para perceberem os resultados (Tay, Kose, Loguercio et al.[11] 2009). Sendo assim, a outra opção é o clareamento em consultório, que em apenas duas sessões pode atingir uma variação de até 8 unidades na escala de cor (Tay, Kose, Loguercio et al. [11] 2009; Bernardon, Sartori, Ballarin, et al. [12] 2010; Strobl, Gutknecht, Franzen, et al. [13] 2010; Kossatz, Martins, Loguercio, et al. [14] 2011; Reis, Dalanhol, Cunha, et al.[15] 2011). Apesar da sua efetividade, é possível estimar, com base em estudos clínicos, que 55 a 90% dos pacientes submetidos a esse procedimento apresentam sensibilidade dental (Haywood[4] 2005; Kihn[16] 2007; Marson, Sensi, Vieira et al.[17] 2008; Tay, Kose, Loguercio et al.[11] 2009; Reis, Dalanhol, Cunha, et al.[15] 2011).

Diferentes alternativas têm sido propostas para minimizar essa sensibilidade (Haywood[4] 2005), dentre elas, podemos citar a redução da concentração de peróxido de hidrogênio, a diminuição da frequência e do tempo de uso do gel clareador (Haywood, Leornard, Nelson et al.[18] 1994; Leonard[19] 1998; Tam[20] 2001; Cardoso, Reis, Loguercio et al.[21] 2006), a utilização de nitrato de potássio para diminuir a transmissão do impulso nervoso da dor (Tay, Kose, Loguercio et al.[11] 2009; Reis, Dalanhol, Cunha, et al.[15] 2011) e a utilização de fluoretos que reduzem a permeabilidade dentinária pela oclusão dos túbulos abertos (Armenio, Fitarelli, Armenio et al.[22] 2008).

Outra alternativa recentemente empregada para minorar a sensibilidade dental causada pelo procedimento clareador, baseou-se na administração de um medicamento anti-inflamatório, ibuprofeno 600 mg (Charakorn, Cabanilla, Wagner et al.[23] 2009), que tem a função de bloquear a produção de prostaglandinas (PGs), mediadores envolvidos no processo inflamatório e diretamente relacionados com a dor pulpar (Hargreaves[24] 2002).

As PGs são formadas a partir da cascata do ácido araquidônico (AA) que tem início após a presença de algum estímulo, como por exemplo, um trauma celular que hidrolisa a fosfolipase A_2 presente nessa membrana e esta catalisa a liberação do AA no citoplasma. Em seguida, o AA se converte em duas vias enzímicas chamadas de lipo-oxigenase e COX, nas quais são produzidos os metabólitos do ácido araquidônico. Pela via da COX são formadas as PGs. A COX existe em, pelo menos, duas isoformas: COX-1 e COX-2 (Suleyman, Demircan, Karagoz[25] 2007). A COX-1 é uma enzima "constitutiva", ou seja, ela está sempre presente no organismo humano, atuando em funções fisiológicas e produz PG I_2 e PG E_2 que mantêm a integridade do sistema gastrointestinal. A COX-2 é uma enzima "induzida", que promove a produção de substâncias álgicas ligadas ao processo inflamatório (Suleyman, Demircan, Karagoz [25] 2007).

Charakorn et al.[23] (2009) utilizou o anti-inflamatório ibuprofeno 600 mg não seletivo para as isoformas da enzima ciclo-oxigenase (COX) administrado em dose única, 30 minutos antes do clareamento em consultório. Comparado com um placebo, seu emprego reduziu apenas a intensidade da sensibilidade imediatamente após o procedimento clareador, não sendo efetivo nas 24 horas seguintes. Os autores[19] atribuíram esse reduzido efeito analgésico à curta meia-vida do medicamento, que é de aproximadamente 2 horas. Talvez o uso continuado do

medicamento pelo período de 48 horas pudesse prevenir ou minimizar a sensibilidade dental. Outra alternativa seria empregar fármacos anti-inflamatórios seletivos para a inibição da COX-2, chamadas de COXIBEs, como o etoricoxibe. Entretanto, não há na literatura nenhum estudo que tenha avaliado o efeito clínico dessas substâncias terapêuticas para a prevenção da sensibilidade dental.

Além do processo inflamatório (Cooper, Bokmeyer, Bowles[6] 1992; Costa. Riehl, Kina et al.[26] 2010), o peróxido de hidrogênio (H_2O_2) e seus subprodutos como ânions superóxido (O^-_2 •) e radicais hidroxila (HO •) causam danos pulpares devido ao estresse oxidativo (Kanno, Ishikawa, Takayanagi et al.[27] 2000), ou seja, o gel utilizado para a realização do clareamento dental aumenta a concentração de espécies reativas de oxigênio (EROs) no interior pulpar causando danos aos componentes celulares (Kanno, Ishikawa, Takayanagi et al.[27] 2000).

As células geram energia reduzindo oxigênio molecular em água. Durante esse processo, formam-se produtos indesejáveis como EROs ou radicais livres que danificam os componentes celulares. Porém, a célula tem um mecanismo de defesa para eliminar esses produtos e o desequilíbrio entre o sistema de geração e eliminação causa o estresse oxidativo (Calabrese, Lodi, Tonon et al.[28] 2005). Em um estudo *in vitro*, Kanno, Shouji, Asou et al.[29] (2003) verificaram que o H_2O_2 30% causou citotoxicidade, apoptose e genotoxicidade nas células de rato P388 via estresse oxidativo. Nesse mesmo estudo, os autores também avaliaram o efeito do antioxidante Narigina em prevenir os danos celulares mencionados. De acordo com os autores, a citotoxicidade e os danos ao DNA induzidos pelo H_2O_2 foi significativamente atenuado pelo uso do antioxidante avaliado. Embora esses resultados sejam provenientes de estudos *in vitro*, eles encorajam estudos clínicos para avaliar o efeito da administração de substâncias antioxidantes na sensibilidade provocada pelo clareamento.

Baseados no exposto, propomo-nos a investigar o efeito de um fármaco anti-inflamatório não seletivo para as isoformas de COX (ibuprofeno 400 mg - administrado de 8 em 8 horas), e de um seletivo para a inibição da isoforma COX-2 (etoricoxibe 60 mg - com dose única diária), assim como de um antioxidante (ácido ascórbico 500 mg - administrado de 8 em 8 horas). Esses medicamentos foram administrados em um período de 48 horas.

2. PROPOSIÇÃO

2.1 PROPOSIÇÕES GERAIS

2.1.1 Avaliar o efeito do anti-inflamatório não seletivo para as isoformas COX (ibuprofeno 400 mg), administrado de 8 em 8 horas, durante 48 horas, no risco absoluto de sensibilidade decorrente da realização da técnica de clareamento dental em consultório;

2.1.2 Avaliar o efeito do anti-inflamatório seletivo para enzima COX-2 (etoricoxibe 60 mg), administrado em dose única diária, durante 48 horas, no risco absoluto de sensibilidade decorrente da realização da técnica de clareamento dental em consultório;

2.1.3 Avaliar o efeito do antioxidante (ácido ascórbico 500 mg), administrado de 8 em 8 horas, durante 48 horas, no risco absoluto de sensibilidade decorrente da realização da técnica de clareamento dental em consultório.

2.2 PROPOSIÇÕES ESPECÍFICAS

2.2.1 Avaliar o efeito do anti-inflamatório não seletivo COX (ibuprofeno 400 mg), administrado de 8 em 8 horas, durante 48 horas, na intensidade da sensibilidade dental após clareamento dental em consultório;

2.2.2 Avaliar o efeito do anti-inflamatório não seletivo COX (ibuprofeno 400 mg), administrado de 8 em 8 horas, durante 48 horas, na magnitude de clareamento alcançado com a técnica de clareamento dental em consultório;

2.2.3 Avaliar o efeito do anti-inflamatório seletivo para a enzima COX-2 (etoricoxibe 60 mg), administrado de 8 em 8 horas, durante 48 horas, na intensidade da sensibilidade dental após clareamento dental em consultório;

2.2.4 Avaliar o efeito do anti-inflamatório seletivo para enzima COX-2 (etoricoxibe 60 mg), administrado de 8 em 8 horas, durante 48 horas, na magnitude de clareamento alcançado com a técnica de clareamento dental em consultório;

2.2.5 Avaliar o efeito do ácido ascórbico 500 mg (vitamina C), administrado de 8 em 8 horas, durante 48 horas, na intensidade da sensibilidade dental após clareamento dental em consultório;

2.2.6 Avaliar o efeito do ácido ascórbico 500 mg (vitamina C), administrado de 8 em 8 horas, durante 48 horas, na magnitude de clareamento alcançado com a técnica de clareamento dental em consultório;

3 MATERIAL E MÉTODOS

Conforme mencionamos no Capítulo 2 escolhemos para esta tese, fazer três experimentos: experimento 1 com ibuprofeno 400 mg; experimento 2 com etoricoxibe 60 mg; e experimento 3 com ácido ascórbico 500 mg (vitamina C), para os quais seguimos a mesma metodologia.

Ressaltamos que nos experimentos não ocorreram diferenças metodológicas expressivas, por isso, escolhemos fazer a descrição detalhada somente para o experimento 1, cf. o item 3.1. Para os experimentos 2 e 3, optamos somente por citar as diferenças essenciais, as quais estão apontadas ao final deste capítulo, cf. item 3.2 – diferenças metodológicas no experimento 2 e o item 3.3 – diferenças metodológicas no experimento 3.

3.1 EXPERIMENTO 1

Este estudo foi aprovado pelo Comissão de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Ponta Grossa (Parecer: 21/2010, protocolo número 17836/2010 – ANEXO A). A descrição do estudo seguiu as normativas do CONSORT (*Consolidated Standards of Reporting Trials*) (Schulz, Altman, Moher[30] 2010). Para participar deste estudo foram selecionados voluntários na cidade de Guarapuava (Paraná, Brasil). Uma semana antes do início do procedimento de clareamento dental, os voluntários assinaram um termo de consentimento livre e esclarecido (Anexo B) e receberam profilaxia com pedra pomes e água em todos os dentes para auxiliar na anamnese detalhada do paciente.

3.1.1 Desenho de Estudo

Este estudo foi um ensaio clínico randomizado, triplo-cego do tipo paralelo controlado por placebo, com taxa de alocação semelhante entre os grupos. O estudo foi realizado na cidade de Guarapuava (Paraná, Brasil), entre janeiro e fevereiro de 2011.

3.1.2 Critérios de Inclusão e Exclusão

Recrutamos os voluntários por meios de comunicação visual, como cartazes e anúncios em rádio e TV, na cidade de Guarapuava (Paraná, Brasil). Para verificar se os mesmos se enquadravam nos critérios de inclusão e exclusão deste estudo, examinamos clinicamente 247 voluntários.

Os critérios adotados para inclusão dos voluntários foram: ser maior de 18 anos, com boa saúde bucal e geral, ter seis dentes anteriores e pré-molares superiores e inferiores livres de lesão cariosa, não ter restaurações nas faces vestibulares; e ainda ter a cor inicial dos dentes C2 ou mais escura, em comparação com a escala de cor Vita Clássica (Vita Lumin, Vita Zahnfabrik, Bad Säckingen, Alemanha) organizada por ordem de luminosidade do mais claro (B1) para o mais escuro (C4) (Figura 1).

Figura 1 – Escala de cor Vita Clássica (Vita Lumin, Vita Zahnfabrik, Bad Säckingen, Alemanha) organizada por ordem de luminosidade

Excluímos os voluntários que já tivessem sido submetidos a algum tipo de procedimento clareador, assim como, os voluntários com sensibilidade dental auto-referida, os que apresentassem dentes com escurecimento severo (manchamento por tetraciclina, fluorose ou endodontia); os que tivessem recessão gengival e hábitos parafuncionais. Excluímos também os fumantes, as grávidas e as

lactantes, assim como, os que exibissem qualquer outro tipo de patologia bucal. Além disso, excluímos os que relataram durante a anamnese, que apresentavam alergia a qualquer um dos medicamentos e alterações sistêmicas, tais como: problemas gástricos, cardíacos, renais e hepáticos e os que fizessem uso contínuo de algum medicamento com ação antioxidante, anti-inflamatória e analgésica.

3.1.3 Cálculo do Tamanho da Amostra

O desfecho primário desse estudo foi avaliar o risco absoluto de sensibilidade dental causado pelo clareamento em consultório. Visto que na literatura (Tay et al.[12] 2009), é relatado que o risco absoluto de sensibilidade dental é de aproximadamente 87% com o produto Whiteness HP Maxx (FGM, Joinville, Santa Catarina, Brasil). No intuito de detectar uma redução de 87% para 41% com um poder de 80% e com um alfa bilateral de 5%, o tamanho amostral mínimo calculado foi de 15 pacientes para cada grupo de estudo.

3.1.4 Randomização, Cegamento e Intervenção

Randomizamos os voluntários em dois grupos. No Grupo controle cada voluntário recebeu seis doses de placebo administradas a cada 8 horas. O placebo foi feito em cápsulas contendo talco farmacêutico livre de lactose (Talco Pharma S M-200, Henrifarma produtos químicos e farmacêuticos LTDA, São Paulo, SP, Brasil). No Grupo experimental**,** os voluntários receberam seis doses do comprimido ibuprofeno 400 mg (Uniprofen, União Química Farm Nacional S/A, Embu-Guaçu, SP, Brasil) administradas a cada 8 horas. Esse medicamento foi entregue aos voluntários, por um pesquisador que não estava envolvido no protocolo da pesquisa, o que impediu que tanto voluntário, quanto operador soubessem acerca de qual medicamento foi usado.

Realizamos a randomização dos voluntários nos dois grupos através de um programa de computador, disponível gratuitamente *online* no sítio http://www.sealedenvelope.com. O processo de aleatorização, realizado por um pesquisador que não estava envolvido, aconteceu somente no momento de iniciar o protocolo, no qual o operador abria o envelope selado que definia o grupo para o qual o voluntário estava alocado. Dessa forma, o estudo foi triplo-cego, no qual nem

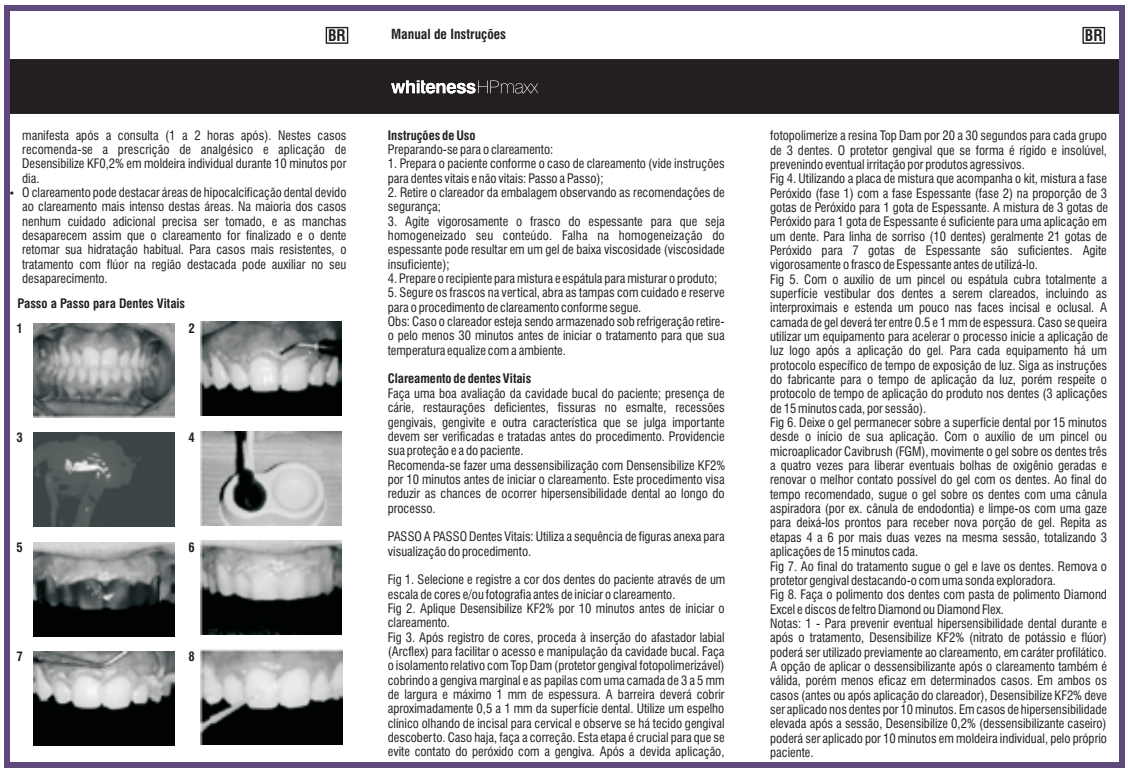
o operador (cirurgião dentista que realizou o clareamento dental), nem o participante e nem o estatístico conheciam a alocação dos grupos.

Todos os medicamentos fornecidos aos voluntários foram retirados de suas embalagens e selados em novas embalagens com nome do voluntário, número da sessão de clareamento e horários de administração. Esse procedimento impediu que o voluntário soubesse qual medicamento estava utilizando. Os medicamentos foram administrados 1 hora antes e até 48 horas após o tratamento. Além disso, os voluntários receberam fichas de avaliação de dor no sentido de relatar informações sobre a sensibilidade dental.

Como forma de manter a adesão ao protocolo e garantir que o medicamento fosse administrado na hora certa, os voluntários receberam ligações e mensagens via celular para lembrá-los dos horários e das anotações nas fichas de avaliação de dor.

3.1.5 Clareamento Dental em Consultório

Antes da aplicação do agente clareador no voluntário utilizamos um afastador (Arcflex, FGM, DENTSCARE LTDA, Joinville, SC, Brasil) para proteção dos lábios, língua e bochechas, seguido de isolamento gengival com a aplicação de resina fotopolimerizável (Top Dam, FGM, DENTSCARE LTDA, Joinville, SC, Brasil). Em cada sessão de clareamento, o gel de peróxido de hidrogênio 35% (Whiteness HP Maxx, FGM, DENTSCARE LTDA, Joinville, SC, Brasil) foi aplicado por 3 vezes de 15 minutos, de acordo com as instruções do fabricante (Figura 2).



BR Manual de Instruções BR

whitenessHPmaxx

manifesta após a consulta (1 a 2 horas após). Nestes casos recomenda-se a prescrição de analgésico e aplicação de Desensibilize KF0,2% em moldeira individual durante 10 minutos por dia.

- O clareamento pode destacar áreas de hipocalcificação dental devido ao clareamento mais intenso destas áreas. Na maioria dos casos nenhum cuidado adicional precisa ser tomado, e as manchas desaparecem assim que o clareamento for finalizado e o dente retomar sua hidratação habitual. Para casos mais resistentes, o tratamento com flúor na região destacada pode auxiliar no seu desaparecimento.

Passo a Passo para Dentes Vitais

1 2 3 4 5 6 7 8

Instruções de Uso
Preparando-se para o clareamento:
1. Prepara o paciente conforme o caso de clareamento (vide instruções para dentes vitais e não vitais: Passo a Passo);
2. Retire o clareador da embalagem observando as recomendações de segurança;
3. Agite vigorosamente o frasco do espessante para que seja homogeneizado seu conteúdo. Falha na homogeneização do espessante pode resultar em um gel de baixa viscosidade (viscosidade insuficiente);
4. Prepare o recipiente para mistura e espátula para misturar o produto;
5. Segure os frascos na vertical, abra as tampas com cuidado e reserve para o procedimento de clareamento conforme segue.
Obs: Caso o clareador esteja sendo armazenado sob refrigeração retire-o pelo menos 30 minutos antes de iniciar o tratamento para que sua temperatura equalize com a ambiente.

Clareamento de dentes Vitais
Faça uma boa avaliação da cavidade bucal do paciente; presença de cárie, restaurações deficientes, fissuras no esmalte, recessões gengivais, gengivite e outra característica que se julga importante devem ser verificadas e tratadas antes do procedimento. Providencie sua proteção e a do paciente.
Recomenda-se fazer uma dessensibilização com Densensibilize KF2% por 10 minutos antes de iniciar o clareamento. Este procedimento visa reduzir as chances de ocorrer hipersensibilidade dental ao longo do processo.

PASSO A PASSO Dentes Vitais: Utiliza a sequência de figuras anexa para visualização do procedimento.

Fig 1. Selecione e registre a cor dos dentes do paciente através de um escala de cores e/ou fotografia antes de iniciar o clareamento.
Fig 2. Aplique Desensibilize KF2% por 10 minutos antes de iniciar o clareamento.
Fig 3. Após registro de cores, proceda à inserção do afastador labial (Arcflex) para facilitar o acesso e manipulação da cavidade bucal. Faça o isolamento relativo com Top Dam (protetor gengival fotopolimerizável) cobrindo a gengiva marginal e as papilas com uma camada de 3 a 5 mm de largura e máximo 1 mm de espessura. A barreira deverá cobrir aproximadamente 0,5 a 1 mm da superfície dental. Utilize um espelho clínico olhando de incisal para cervical e observe se há tecido gengival descoberto. Caso haja, faça a correção. Esta etapa é crucial para que se evite contato do peróxido com a gengiva. Após a devida aplicação, fotopolimerize a resina Top Dam por 20 a 30 segundos para cada grupo de 3 dentes. O protetor gengival que se forma é rígido e insolúvel, prevenindo eventual irritação por produtos agressivos.
Fig 4. Utilizando a placa de mistura que acompanha o kit, mistura a fase Peróxido (fase 1) com a fase Espessante (fase 2) na proporção de 3 gotas de Peróxido para 1 gota de Espessante. A mistura de 3 gotas de Peróxido para 1 gota de Espessante é suficiente para uma aplicação em um dente. Para linha de sorriso (10 dentes) geralmente 21 gotas de Peróxido para 7 gotas de Espessante são suficientes. Agite vigorosamente o frasco de Espessante antes de utilizá-lo.
Fig 5. Com o auxílio de um pincel ou espátula cubra totalmente a superfície vestibular dos dentes a serem clareados, incluindo as interproximais e estenda um pouco nas faces incisal e oclusal. A camada de gel deverá ter entre 0.5 e 1 mm de espessura. Caso se queira utilizar um equipamento para acelerar o processo inicie a aplicação de luz logo após a aplicação do gel. Para cada equipamento há um protocolo específico de tempo de exposição de luz. Siga as instruções do fabricante para o tempo de aplicação da luz, porém respeite o protocolo de tempo de aplicação do produto nos dentes (3 aplicações de 15 minutos cada, por sessão).
Fig 6. Deixe o gel permanecer sobre a superfície dental por 15 minutos desde o início de sua aplicação. Com o auxílio de um pincel ou microaplicador Cavibrush (FGM), movimente o gel sobre os dentes três a quatro vezes para liberar eventuais bolhas de oxigênio geradas e renovar o melhor contato possível do gel com os dentes. Ao final do tempo recomendado, sugue o gel sobre os dentes com uma cânula aspiradora (por ex. cânula de endodontia) e limpe-os com uma gaze para deixá-los prontos para receber nova porção de gel. Repita as etapas 4 a 6 por mais duas vezes na mesma sessão, totalizando 3 aplicações de 15 minutos cada.
Fig 7. Ao final do tratamento sugue o gel e lave os dentes. Remova o protetor gengival destacando-o com uma sonda exploradora.
Fig 8. Faça o polimento dos dentes com pasta de polimento Diamond Excel e discos de feltro Diamond ou Diamond Flex.
Notas: 1 - Para prevenir eventual hipersensibilidade dental durante e após o tratamento, Desensibilize KF2% (nitrato de potássio e flúor) poderá ser utilizado previamente ao clareamento, em caráter profilático. A opção de aplicar o dessensibilizante após o clareamento também é válida, porém menos eficaz em determinados casos. Em ambos os casos (antes ou após aplicação do clareador), Desensibilize KF2% deve ser aplicado nos dentes por 10 minutos. Em casos de hipersensibilidade elevada após a sessão, Desensibilize 0,2% (dessensibilizante caseiro) poderá ser aplicado por 10 minutos em moldeira individual, pelo próprio paciente.

Figura 2 - Instruções do fabricante para o uso do o gel de peróxido de hidrogênio 35% (Whiteness HP Maxx, FGM, DENTSCARE LTDA, Joinville, SC, Brasil)

O clareamento foi realizado na superfície vestibular dos dentes das arcadas superior e inferior até os primeiros pré-molares, por um único operador. Este procedimento foi repetido depois de uma semana, seguindo o mesmo protocolo (3 vezes de 15 minutos). Portanto, realizamos duas sessões de clareamento em todos os voluntários dos dois grupos. Orientamos os voluntários a não ingerirem alimentos ácidos e pigmentados, nem bebidas como café, chá e vinho tinto durante todo o experimento.

3.1.6 Avaliação da Intensidade de Sensibilidade Dental

O efeito do medicamento foi avaliado pelo voluntário nas duas sessões de clareamento dental, em três momentos diferentes, durante o procedimento em até 1 hora, nas 24 horas e nas 48 horas após a realização do clareamento. Para os dentes das arcadas superior e inferior, utilizamos a escala verbal de 5 pontos, em que 0 = indica nenhuma sensibilidade, 1 = leve, 2 = moderada, 3 = considerável e 4 = severa (Tay, Kose, Loguercio et al.[11] 2009; Reis,

Dalanhol, Cunha, et al.[15] 2011). Solicitamos que o voluntário informasse numericamente o escore da sua sensibilidade em cada período citado.

Além dessa escala, empregamos também a escala visual analógica (EVA) que consiste numa linha de 10 cm com os dois extremos fechados (Jensen, Karolpy, Braver[30] 1986). Um dos extremos apresenta a indicação “sem dor” e no outro “dor insuportável”. Orientamos cada voluntário para marcar, com um único traço vertical, o ponto que melhor correspondesse à sua dor no momento da avaliação. A determinação do valor da intensidade da dor foi feita medindo a distância em milímetros entre o ponto “sem dor” até a marca realizada pelo voluntário.

A avaliação da sensibilidade foi registrada em fichas separadas para os dentes superiores e inferiores. O voluntário também recebeu instruções para realizar anotações sobre qual ou quais dentes sentia sensibilidade e os horários em que esta ocorria. Utilizamos o valor do pior *score* informado nas duas escalas de dor, nos 3 períodos, para a realização do tratamento estatístico.

3.1.7 Avaliação da Alteração de Cor

Fizemos a avaliação de cor antes do início e 30 dias após o tratamento clareador, utilizando avaliação subjetiva com escala de cor Vita Clássica (Vita Lumin, Vita Zahnfabrik, Bad Säckingen, Alemanha) (Figura 1) e avaliação objetiva com espectrofotômetro Vita Easy Shade (Vita Zahnfabrik, Bad Säckingen, Alemanha) (Figura 3).

Figura 3 – Avaliação objetiva com o Espectrofotômetro Vita Easy Shade (Vita Zahnfabrik, Bad Säckingen, Alemanha)

A área escolhida para avaliação da cor foi o terço médio da face vestibular do incisivo central superior direito, de acordo com as orientações da *American Dental Association* (ADA)[32] (2001). Para fins de calibração, dois examinadores avaliaram cinco voluntários em uma mesma sala, sob a mesma iluminação. Os avaliadores deveriam apresentar concordância de pelo menos 85% (teste de Kappa) antes de iniciar o estudo (Reis et al.[13] 2011). Esses voluntários não fizeram parte da amostra, participaram apenas do estudo piloto.

A escala de cor Vita Clássica (Vita Lumin, Vita Zahnfabrik, Bad Säckingen, Alemanha) foi organizada por ordem decrescente de luminosidade, do maior (B1) para o menor (C4). Essa escala não apresenta valores numéricos, assim atribuímos valores contínuos de 1 a 16 para ranqueamento. Para critério de inclusão, o valor mínimo da cor deveria ser C2 com valor igual a 7 na escala de cor. A variação da alteração da cor inicial e após 30 dias do tratamento clareador pelo método subjetivo foi calculada pela diferença entre a unidade de cor da escala Vita inicial (UCV_0) subtraída da unidade de cor da escala Vita final (UCV_1), através da seguinte fórmula:

$$\Delta UCV = UCV_0 - UCV_1$$

Para a avaliação objetiva da cor utilizando o espectrofotômetro, primeiramente foram moldados os dentes superiores anteriores de canino a canino, com silicone de adição de consistência pesada (Express, 3M ESPE, St. Paul, Minessota, EUA) para a realização de uma matriz. Essa matriz foi perfurada na altura do terço médio do incisivo central direito com ajuda de um bisturi circular de 6 mm de diâmetro (Biopsy Punch, Miltex, York, Pensilvânia, EUA), similar ao diâmetro da ponta do espectrofotômetro (Marson et al.[11] 2008; Cardoso et al.[17] 2006) (Figura 4) que serviu como guia para padronizar as leituras.

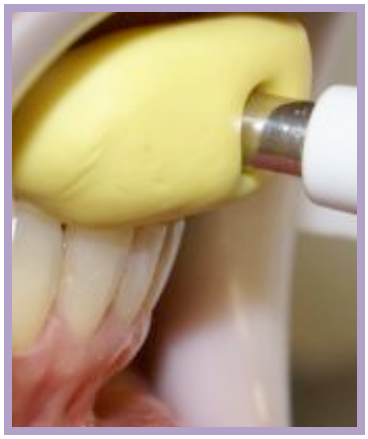


Figura 4 – Moldagem com silicone de adição de consistência pesada pesada (Express, 3M ESPE, St. Paul, Minessota, EUA) servindo de guia para padronização das leituras de cor com o espectrofotômetro

O espectrofotômetro Easy Shade (Vita Zahnfabrik, Bad Säckingen, Alemanha) indicou os seguintes parâmetros de cor: L*, a* e b*, onde L* indica luminosidade, a* variação de cor na direção verde - vermelho e b* variação de cor na direção amarelo - azul (Figura 5). A variação de cor (ΔE) registrada inicialmente e após o procedimento clareador foi calculada pela seguinte fórmula (Marson et al.[11] 2008; Cardoso et al.[17] 2006):

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

Figura 5 - O espectrofotômetro Easy Shade (Vita Zahnfabrik, Bad Säckingen, Alemanha), indicando os parâmetros de cor: L*, a* e b*, onde L* indica luminosidade, a* variação de cor na direção verde-vermelho e b* variação de cor na direção amarelo-azul

3.1.8 Planejamento Experimental Estatístico

A análise seguiu o protocolo de intenção de tratar envolvendo todos os voluntários que foram aleatoriamente selecionados (Schulz, Altman, Moher[26] 2010). O estatístico foi cegado para estudar os grupos. O acordo entre examinadores para a avaliação objetiva foi através do teste de *Kappa*. Comparamos o desfecho primário, *risco absoluto de sensibilidade dental,* usando o teste exato de Fisher (alfa = 5%). Calculamos também o risco relativo e o intervalo de confiança para o tamanho do efeito.

Analisamos estatisticamente a intensidade da sensibilidade dental (objetivo secundário). Calculamos média, mediana, desvio-padrão e os valores mínimo e máximo das duas escalas de dor. Plotamos os conjuntos de dados da intensidade de sensibilidade dental da Escala Visual Analógica (EVA) em histogramas e os inspecionamos para distribuições normais. Alguns dados não tinham distribuição normal por isso, utilizamos testes estatísticos não paramétricos para comparar os diferentes tratamentos. As análises estatísticas das duas escalas de dor, comparando os grupos em cada período de avaliação foram realizadas utilizando o teste de Mann-Whitney. As comparações entre os tempos dentro do mesmo grupo foram realizadas utilizando o teste de Friedman.

Os dados do grupo placebo foram usados para comparar o risco absoluto e a intensidade da sensibilidade entre as arcadas superior e inferior. Para o primeiro, foi utilizado o teste exato de Fisher (alfa de 5%). Para este último, foi utilizado o teste de Mann-Whitney com o mesmo nível de significância.

Avaliamos a alteração da cor, o segundo desfecho secundário, para verificar o efeito do uso pré-operatório do medicamento ibuprofeno 400 mg na magnitude do clareamento alcançada com a técnica de clareamento dental em consultório. Para as avaliações objetiva e subjetiva da alteração da cor calculamos a média e o desvio-padrão da variação da cor inicial e após 30 dias do clareamento dental para cada grupo e os dados foram avaliados pelo teste *t* de *Student*. Em todos os testes estatísticos fixamos o nível de significância em alfa de 5%.

3.2 DIFERENÇAS METODOLÓGICAS NO EXPERIMENTO 2

3.2.1 Desenho de Estudo

Realizamos o estudo entre agosto e dezembro de 2010.

3.2.2 Randomização, Cegamento e Intervenção

Randomizamos os voluntários em dois grupos: controle e experimental. No Grupo controle cada voluntário recebeu duas doses de placebo administradas a cada 24 horas. O placebo foi feito em cápsulas contendo talco farmacêutico (Talco Pharma S M-200, Henrifarma produtos químicos e farmacêuticos LTDA, São Paulo, SP, Brasil) livre de lactose. No Grupo experimental**,** os voluntários receberam duas doses do comprimido etoricoxibe 60 mg (Arcoxia, Merck & Co., Inc., Whitehouse Station, NJ, EUA) administradas a cada 24 horas.

3.2.3 Avaliação da Intensidade de Sensibilidade Dental

Nesta pesquisa também utilizamos a escala numérica de 101 pontos, onde o voluntário escolheu um número entre 0 e 100 que melhor representasse a sua dor, sendo que 0 corresponde a classificação “sem dor” e 100 a classificação “dor insuportável”.

Registramos a avaliação da sensibilidade somente para os dentes da arcada superior.

3.2.4 Planejamento Experimental Estatístico

Avaliamos a alteração da cor (desfecho secundário), para verificar o efeito do uso pré-operatório do medicamento etoricoxibe 60 mg na magnitude de clareamento alcançada com a técnica de clareamento dental em consultório. Para avaliação objetiva e subjetiva da alteração da cor calculamos a média e o desvio-padrão da variação de cor inicial e após 30 dias do clareamento dental para cada grupo. Submetemos os dados da avaliação subjetiva à análise de medidas repetidas ANOVA 2 critérios e os da avaliação objetiva à estatística teste *t* de *Student*. Usamos a análise post-hoc (teste de Tukey) para fazer comparações entre pares.

3.3 DIFERENÇAS METODOLÓGICAS NO EXPERIMENTO 3

3.3.1 Desenho de Estudo

Realizamos este estudo entre maio e junho de 2012.

3.3.2 Cálculo do Tamanho da Amostra

O desfecho primário deste estudo foi avaliar o risco absoluto de sensibilidade dental causado pelo clareamento de consultório. Na literatura (Tay et al.[12] 2009) é reportado que o risco absoluto de sensibilidade dental é de aproximadamente 90% com o produto Whiteness HP Maxx (FGM, Joinville, Santa Catarina, Brasil). No intuito de detectar uma redução de 90% para 40% com um poder de 80% e com um alfa bilateral de 5%, o tamanho amostral mínimo calculado foi de 19 voluntários para cada grupo de estudo, sendo que o Grupo experimental apresentou 20 voluntários e o grupo controle 19 voluntários.

3.3.3 Randomização, Cegamento e Intervenção

Randomizamos os voluntários foram randomizados em dois grupos: controle e experimental. No Grupo controle cada voluntário recebeu seis doses de placebo administradas a cada 8 horas. O placebo foi feito em cápsulas contendo talco farmacêutico (Talco Pharma S M-200, Henrifarma produtos químicos e farmacêuticos LTDA, São Paulo, SP, Brasil) livre de lactose. No Grupo experimental**,** os voluntários receberam seis doses do comprimido ácido ascórbico 500 mg (Citroplex Vitamina C, Neo Química, Anápolis, GO, Brasil) administradas a cada 8 horas.

3.3.4 Avaliação da Intensidade de Sensibilidade Dental

A avaliação ocorreu da mesma maneira que para o Experimento 1, com a diferença que foram avaliados somente os dentes da arcada superior.

4 ARTIGOS

4.1 The effect of perioperative ibuprofen use on tooth sensitivity caused by in-office bleaching.

4.2 Perioperative use of an anti-inflammatory drug on tooth sensitivity caused by in-office bleaching: a randomized, triple-blind clinical trial.

4.3 Administration of ascorbic acid to prevent bleaching-induced tooth sensitivity: a randomized triple-blind clinical trial.

4.1 The effect of perioperative ibuprofen use on tooth sensitivity caused by in-office bleaching.

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The Effect of Perioperative Ibuprofen Use on Tooth Sensitivity Caused by In-Office Bleaching

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A Loguercio • A Reis

Clinical Relevance

The anti-inflammatory drug ibuprofen reduced the intensity of tooth sensitivity up to one hour after in-office bleaching treatment.

SUMMARY

Objective: This study determined the effect of the administration of perioperative ibuprofen 400 mg on tooth sensitivity caused by in-office bleaching.

Methods: A triple-blind, parallel-design, randomized clinical trial was conducted on 30 adults who received placebo or ibuprofen before and after bleaching. The drugs were administered three times per day for 48 hours; the first dose was given one hour prior to the bleaching treatment. Two bleaching sessions with 35% hydrogen peroxide gel were performed with a one-week interval. Tooth sensitivity was recorded on two scales: visual analogue and five-point verbal rating scale up to 48 hours after bleaching. The shade evaluation was performed with a visual shade guide and spectrophotometer, before and 30 days after bleaching. The absolute risk of tooth sensitivity and its intensity were evaluated by Fisher exact and Mann-Whitney tests, respectively. The shade changes were evaluated by Student's t-test.

Results: Both groups showed similar absolute risk of tooth sensitivity ($p>0.05$). Lower tooth sensitivity was observed in the experimental group only up to one hour postbleaching ($p=0.04$). Similar tooth sensitivity was observed in the other periods of time.

Conclusion: The perioperative use of the anti-inflammatory ibuprofen was not able to avoid tooth sensitivity but reduced its intensity up to one hour after bleaching.

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INTRODUCTION

The desire for whiter teeth has made tooth bleaching one of the most sought-after cosmetic procedures in dentistry. Available bleaching modalities include dentist-prescribed at-home bleaching and dentist-supervised in-office bleaching.[1] Even though the at-home bleaching system is the most frequently recommended treatment for vital teeth, some patients do not want to use a bleaching tray or do not want to wait two to three weeks to see the results of the treatment.[2] Thus, another bleaching option, the in-office bleaching procedure, is often requested.

However, the in-office procedure using 35% hydrogen peroxide has a long history of tooth sensitivity and gingival irritation.[2,3] Incidence levels of tooth sensitivity have been reported to be as high as 87%,[4-6] which seems to result from the easy passage of the peroxide through the enamel and dentin to the pulp,[7] causing pulpal damage with inflammation.[8] Pulp tissue damage caused by dental bleaching is likely to lead to the release of cell-derived factors, such as adenosine triphosphate[9] and prostaglandins, which excite or sensitize pulpal nociceptors.[10]

The use of desensitizing agents such as fluorides and potassium nitrate before or after bleaching was capable of reducing the experience of tooth sensitivity during the bleaching treatment.[2,5,11,12] However, this approach adds another step to the bleaching protocol, which is against the clinicians' preference for simplification. Another approach recently investigated was the preoperative use of ibuprofen,[13] which is a nonsteroidal anti-inflammatory drug capable of blocking the cyclooxygenase (COX) pathway.[14] Although this clinical alternative seemed promising, the preoperative use of a single dose of ibuprofen 400 mg before the in-office bleaching protocol was shown to reduce tooth sensitivity only during but not after the treatment period.[13] As mentioned by the authors of the previous study,[13] the half-life elimination of ibuprofen is two to four hours, and thus, more than one dose may be required to keep the ibuprofen serum level sufficiently high for optimum analgesic effect.

Therefore, the current study tested the primary hypothesis that the preventive and perioperative use of ibuprofen 400 mg for 48 hours, starting one hour before the bleaching session, would reduce the absolute risk of tooth sensitivity. A secondary hypothesis tested was that the use of this anti-inflammatory drug would reduce the intensity of sensitivity without affecting the bleaching efficacy and shade change.

MATERIALS AND METHODS

This clinical investigation was approved (protocol No. 17836/2010) by the scientific review committee and the committee for the protection of human subjects of the local university. The experimental design followed the Consolidated Standards of Reporting Trials statement.[15] Based on preestablished criteria, 30 volunteers from the city of Guarapuava, Paraná, Brazil were selected for this study and signed a term of free and informed consent to participate. Two weeks before the bleaching procedures, all the volunteers received a dental screening and dental prophylaxis using a rubber cup with pumice and water slurry.

Study Design

This was a randomized, placebo-controlled, triple-blind, parallel-group clinical trial, with an equal allocation rate to receive one of two treatments. The study took place in the clinic of the Brazilian Association of Dentistry in Guarapuava, Paraná, from January 2011 to February 2011.

Inclusion and Exclusion Criteria

Patients included in this 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. A total of 247 participants were examined, in a dental chair, to check if they met the inclusion and exclusion criteria (Figure 1). The participants should have at least eight maxillary and mandibular anterior teeth that were caries free and without restorations on the labial surfaces. The central incisors were C2 or darker as judged by comparison with a value-oriented shade guide (Vita Lumin, Vita Zahnfabrik, Bad Säckingen, Germany). Participants who had undergone tooth-whitening procedures, had preexisting anterior restorations, were pregnant/lactating, had severe internal tooth discoloration (tetracycline stains, fluorosis or pulpless teeth), had bruxism habits, or had any other pathology that could cause sensitivity (such as recession or dentin exposure) were excluded from this study to minimize confounding experimental variables or side effects from bleaching. Participants who reported a history of 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.

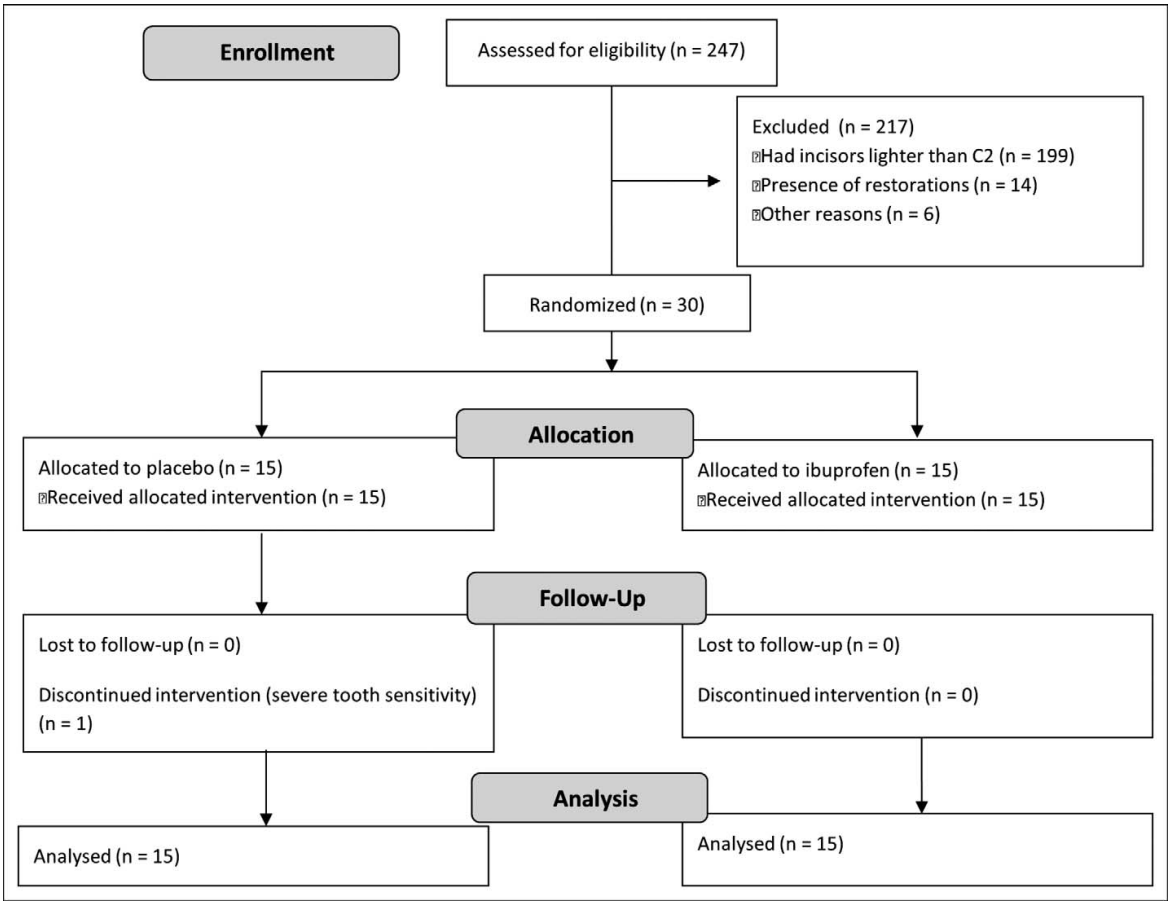

Figure 1. *Flow diagram of the clinical trial including detailed information on the excluded participant.*

Sample Size Calculation

The primary outcome of this study was the absolute risk of tooth sensitivity. This risk was reported to be approximately 87% for the bleaching product Whiteness HP Maxx (FGM, Joinville, SC, Brazil).[4] Thus, 30 patients were required to have an 80% chance of detecting, as significant at the two-sided 5% level, a decrease in the primary outcome measure from 87% in the control group to 41% in the experimental group.

Study Intervention

Participants were randomly stratified by sex into the placebo and ibuprofen groups. The randomization process was performed by computer-generated tables by a third person not involved in the research protocol. Details of the allocated groups were recorded on cards contained in sequentially numbered, opaque, sealed envelopes. These cards were prepared by a third person not involved in any of the phases of the clinical trial. Once the participant was eligible for the procedure and completed all baseline assessments, the allocation assignment was revealed by opening this envelope by this third person. Neither the participant nor the operator knew the group allocation, with both being blinded to the protocol.

The participants from the placebo group received a placebo (Talco pharma S M-200 Henrifarma Prod Quím Farm LTD, São Paulo, SP, Brazil), and participants from the ibuprofen group received a dose of nonsteroidal anti-inflammatory ibuprofen 400 mg (Uniprofen, União Quím Farm Nacional S/A, Embu-Guaçu, SP, Brazil). All participants were

watched to ensure that they took the drugs or placebo one hour prior to treatment. Other doses of placebo or ibuprofen (400 mg) were administered every eight hours after the first dose during a period of 48 hours. When it was time for the participants to take the other doses of medicine and placebo, the research assistant called all patients to remind them to take the drugs. This procedure was done to increase adherence to the protocol.

The gingival tissue of the teeth to be bleached was isolated from the bleaching agent using a light-polymerized resin dam (Top Dam, FGM, Joinville, SC, Brazil). The 35% hydrogen peroxide 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. All participants were instructed to brush their teeth at least three times a day using fluoridated toothpaste (Sorriso Fresh, Colgate-Palmolive, São Paulo, SP, Brazil).

Shade Evaluation

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

For the subjective examination, the 16 shade guide 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, the changes were treated as though they represented a continuous and approximately linear ranking for the purpose of analysis. The measurement area of interest for shade matching was the middle one third of the facial surface of the anterior central incisor. For calibration purposes, five patients who were not included in the sample because they were used in the pilot study participated in the training phase of this study. The two examiners, blinded to the allocation assignment, scheduled these patients 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 statistics) before beginning the study evaluation.[5] The shade comparison before and after treatment is given by the difference between the baseline and 30-day shades (ΔSGU).

For the objective evaluation, a preliminary impression of the maxillary arch was made using dense silicone Adsil (Vigodent S/A Ind Com, Rio de Janeiro, RJ, Brazil). The impression was extended to the maxillary 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 metal device with well-formed borders and radius of 3 mm.[3,16] The measurement was made by only one operator, in all 30 patients, using Vita Easyshade (Easyshade, Vident) before and 30 days after the bleaching therapy. The shade was determined using the parameters of the Easyshade device, which 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 is given by the differences between the two shades (ΔE),[3,16] which is calculated using the formula $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Tooth Sensitivity Evaluation

The patients recorded their perception of tooth sensitivity 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*)[4,5] and a visual analog scale[17-19] (using a 10-cm horizontal line with words *no pain* at one end and *worst pain* at the opposite end) were used in this study. The subjects were asked to record whether they experienced sensitivity during the treatment up to one hour after the bleaching, from one hour to 24 hours and from 24 hours to 48 hours after bleaching, individually, for both maxillary and mandibular arches. As two bleaching sessions were performed, the worst score/numerical value obtained in both bleaching sessions was considered for statistical purposes. The values were arranged into two categories: overall percentage of patients who reported tooth sensitivity at least once during treatment, regardless of the assessment point (absolute risk of tooth sensitivity), and tooth sensitivity intensity at each of the assessment points. These values were computed for both the maxillary and mandibular arches.

Table 1: *Comparison of the Number of Patients Who Experienced Tooth Sensitivity on the Maxillary and Mandibular Arches During the Bleaching Regimen in Both Groups Along With Absolute and Relative Risks*[a]

Treatment	Maxillary Arch				Mandibular Arch			
	Tooth Sensitivity (No. of Participants)		Absolute Risk (95% CI)	Relative Risk (95% CI)	Tooth Sensitivity (No. Participants)		Absolute Risk (95% CI)	Relative Risk (95% CI)
	Yes	No			Yes	No		
Placebo	12	3	80 (55-93)	0.92 (0.67-1.3)	14	1	93 (70-98)	1.16 (0.87-1.6)
Ibuprofen	13	2	87 (62-96)		12	3	80 (54-93)	

[a] *Fisher test,* p=*1.0 for maxilla and* p=*0.59 for mandibular arches.*

Statistical Analysis

The analysis followed the intention-to-treat protocol and involved all participants who were randomly assigned.[15] The statistician was blinded to the study groups. The agreement between examiners' objective evaluation was evaluated using the kappa statistics. The primary outcome of absolute risk of tooth sensitivity was compared using Fisher exact test (α=5%). The relative risk and the confidence interval for the effect size were calculated.

Tooth sensitivity intensity (secondary outcome) was also statistically analyzed. The mean/median and standard deviation/interquartile range of the two pain scales were calculated. The data sets of tooth sensitivity intensity were plotted on histograms and inspected for normal distributions. Some data did not appear to be normally distributed. Therefore, nonparametric statistical tests were used to compare the various treatments. Statistical analyses of two pain scales comparing the two groups at the three different assessment points were 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%.

The data from the placebo group were used to compare the absolute risk of tooth sensitivity and its intensity between the maxillary and mandibular arches. For the former, Fisher exact test at a level of 5% was used. For the latter, the Mann-Whitney U-test was used at the same level of significance.

Color change, another secondary endpoint, was used to assess the efficacy of the bleaching treatment associated with the perioperative use of ibuprofen. The ΔSGU, ΔL, Δa, Δb, and ΔE values of both groups were evaluated by Student *t*-test. In all statistical tests, the significance level was set at alpha of 5%.

RESULTS

The mean age (years) of the participants in this study was similar between the groups (placebo: 26.4 $\pm$ 6.8, and ibuprofen: 32.9 $\pm$ 9.9 years). Fifty-three and 33% of the participants from the placebo and ibuprofen groups were male. Figure 1 depicts the participant flow diagram in the different phases of the study design.

Tooth Sensitivity

The data from 30 patients were used in this study, following the intention-to-treat analysis. In regard to the absolute risk of tooth sensitivity (primary outcome), no significant difference was observed between groups, as seen in Table 1 (p=1.00 for maxilla and p=0.59 for mandibular arches). The relative risk, along with the 95% confidence interval, is also evidence that the use of the experimental drug had no effect on the tooth sensitivity reduction.

In the comparison between the dental arches, no significant difference was detected for both groups, with regard to the absolute risk of tooth sensitivity ($p>0.05$) and tooth intensity ($p>0.05$).

Most of the tooth sensitivity complaints occurred within the first 24 hours ($p<0.001$), and none of the participants experienced pain after 24 hours. When using the visual analogue scale, the tooth sensitivity intensity (Table 2) was less intense for ibuprofen than the placebo group in both arches only up to one hour (p=0.04 for maxillary and p=0.008 for mandibular arches). This was also observed for the five-point verbal rating scale in the mandibular arch (p=0.006), but not in the maxillary arch, which showed no statistical difference between groups (p=0.06).

Significant whitening was observed in both study groups by means of the subjective and objective evaluation methods ($p<0.001$). Whitening of approximately 5.3 and 4.3 shade guide units was detected for placebo and ibuprofen groups, respectively (Table 3), and a variation of 7.8 to 5.9 in the ΔE was observed for the placebo and ibuprofen groups, respectively. The results of the subjective (visual shade guide) and the objective evaluation (spectro-

Table 2: *Comparison of Tooth Sensitivity Intensity Experienced on the Maxillary and Mandibular Anterior Teeth by Patients From the Treatment Groups at Different Assessment Points Using the Pain Scales*[a]

	Five-Point Verbal Rating Scale (0-4)[b]				Visual Analog Scale (0-10)[c]			
	Maxillary Arch		Mandibular Arch		Maxillary Arch		Mandibular Arch	
	Placebo	Ibuprofen	Placebo	Ibuprofen	Placebo	Ibuprofen	Placebo	Ibuprofen
Up to 1 hour	2 (1/3)^{aA}	1 (0/2)^{aA}	3 (1/4)^{aA}	1 (0/2)^{bA}	3.3 ± 2.9^{aA}	1.5 ± 2.0^{bA}	4.3 ± 3.6^{aA}	1.5 ± 2.4^{bA}
1 hour to 24 hours	2 (0/2)^{aB}	2 (1/3)^{aA}	2 (0/3)^{aB}	2 (1/3)^{aB}	2.4 ± 2.7^{aB}	3.0 ± 2.8^{aB}	3.7 ± 3.6^{aA}	3.2 ± 3.0^{aB}
24 hours to 48 hours	0 (0/0)^{aC}	0 (0/0)^{aB}	0 (0/0)^{aC}	0 (0/0)^{aC}	0.0 ± 0.0^{aC}	0.0 ± 0.0^{aC}	0.0 ± 0.0^{aB}	0.0 ± 0.0^{aC}

[a] *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 superscript lowercase letters. For each treatment, the three periods were compared with the Friedman test (α=0.05), and differences are represented by different superscript uppercase letters.*
[b] *Medians (first/third interquartile) values.*
[c] *Means and standard deviations.*

photometer) matched the hypothesis of equality between the groups after bleaching ($p \approx 0.6$ for both methods).

DISCUSSION

It has been reported that tooth-whitening solution applied to human mandibular incisors was shown to cause structural damage to the pulp, such as disruption of the odontoblast layer and the production of inflammatory infiltrates.[8] Tissue damage triggers the creation of bradykinin[20] and prostaglandins.[21,22] Each of these compounds either activates or sensitizes nociceptors and causes pain.[20,21]

The category of prostaglandins, which has been known to play a critical role in the pathogenesis of pulpal disease, involves the COX pathway. There are at least two variants of COX, the COX-1, which is involved in physiological functions and inducible COX-2, which is believed to be involved in the inflammatory response[14] and has been responsible for the production of prostaglandins mediating inflammation and pain due to pulp inflammation.[21]

Table 3: *Change Between Baseline and 30-Day Assessment (Means and Standard Deviations) for ΔSGU, ΔL, Δa, Δb, and ΔE for the Two Treatment Groups*[a] *for the Maxillary Arch*

	Placebo	Ibuprofen
Subjective evaluation (delta visual shade guide)		
Δ shade guide units	5.3 ± 1.9 A	4.3 ± 2.7 A
Objective evaluation (spectrophotometer)		
CIELab		
ΔL	2.2 ± 1.6 A	0.7 ± 4.4 A
Δa	−1.3 ± 1.4 A	−0.7 ± 0.8 A
Δb	−7.0 ± 3.2 A	−3.9 ± 1.6 B
ΔE	7.8 ± 3.3 A	5.9 ± 2.1 A

[a] *Comparisons are valid only within rows. Similar uppercase letters indicate statistically similar means (Student t-test, α=0.05).*

Ibuprofen is a nonsteroidal anti-inflammatory drug, and its mechanism of action results from acetylating the COX enzyme, which in turn inhibits the synthesis of prostaglandins.[22] However, contrary to one's previous expectation, the use of a nonsteroidal anti-inflammatory drug (ibuprofen 400 mg) in a preventive approach was not capable of avoiding the tooth sensitivity arising from bleaching. The lack of efficacy of ibuprofen in preventing tooth sensitivity could be that several other inflammatory mediators,[22] apart from COX-1 and COX-2, are probably involved in the inflammatory reaction in the pulp tissue that leads to pain and symptoms of neurogenic inflammation.

For instance, bradykinin[20] and substance-P have long been known to be involved in the process of pulp pain and inflammation.[23] Unfortunately, ibuprofen cannot prevent the production of these mediators. If many mediators act synergistically to produce both pain and inflammatory reaction after bleaching, an anti-inflammatory drug that inhibits several initial inflammatory events, such as the glucocorticoids,[24] may be more effective in reducing tooth sensitivity arising from bleaching. Chrousos and others[25] reported that some side effects have been reported with short-term oral glucocorticoid therapy such as insomnia, mild mood changes, stomach upset, facial flushing, and weight gain; however, these events are not often observed when glucocorticoid therapy is performed in young, healthy adults, and it is considered a relatively safe procedure. This strategy should be a focus of future clinical investigations that attempt to reduce tooth sensitivity caused by bleaching.

Dental pulp is densely innervated with sensory afferents with conduction velocities in the A"β, Aδ, and C-fiber range.[26] Thus, hydrogen peroxide may cause direct cellular damage to nerve cells via free radicals and other reactive oxygen species[27] produc-

ing lipid peroxidation of membrane protein and nucleic acid oxidation.[28,29] If this is the cause of tooth sensitivity, the use of an anti-inflammatory drug, such the one investigated in the present study, would not reduce the pain experience.

It is worth mentioning, however, that the intensity of tooth sensitivity in the ibuprofen group was lower during the first hour after bleaching in the present investigation, similar to the findings observed in the study by Charakorn and others.[13] This means that this medicine can be used to reduce the intensity of tooth sensitivity only during and immediately after the bleaching session.

However, no significant difference between groups was observed in the following assessment points. In Charakorn and others' study,[13] this finding was explained by the decreasing amount of ibuprofen in patients' serum as time passes. However, the results of the present investigation do not support this hypothesis since ibuprofen was administered in six doses during a period of 48 hours after bleaching and not in a single dose, as in Charakorn and others' study.[13] This finding suggests that the prostaglandins produced by COX[14] probably occur in the first hours after bleaching. As time goes by, other inflammatory mediators, not inhibited by the ibuprofen, may be expressed and trigger the tooth sensitivity.

Costa and others[8] in a histological pulp evaluation after bleaching, showed notable damage to the pulp tissue of mandibular incisors but not premolars. In a literature review, Haywood[30] reported that tooth sensitivity from bleaching usually affects the smaller teeth, such as the maxillary lateral incisors and the mandibular incisors, although the present clinical study found no significant difference in tooth sensitivity intensity or in the absolute risk of tooth sensitivity between dental arches; in other words, the tooth sensitivity may be related to the thickness of the hard dental tissues rather than to the arch itself. Further studies should be conducted to identify the most painful teeth and follow up their vitality in the long run after bleaching, suggesting that tooth sensitivity intensity may be a reliable signal of significant changes in the pulp tissue.

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 baseline values (Table 3). It is difficult to make a comparison of shade change after in-office bleaching with studies in the existing literature, because of the different methods of measurement (shade guides and spectrophotometer) and different units of measurement (CIELab system, shade guide units, etc) used. However, studies that used 35% hydrogen peroxide and reported their results in shade guide units usually observed an overall shade change of five to eight shade guide units after two bleaching sessions,[4,5,6] which is in agreement with the results of the present investigation.

Finally, one could not ignore that the small sample size of this study is a clear limitation of the present study. The study was designed to find a high effect size, that is, reduction in 50% of the tooth sensitivity among participants in the experimental group. Thus, it can be concluded that an effect as large as this was not observed, but one cannot rule out the fact that smaller effect sizes do exist. Conducting the same experimental design using larger sample sizes should be encouraged to rule out this hypothesis. Moreover, the selected sample was mainly composed of young participants, which also limits the ability to generalize for older adults.

CONCLUSION

The perioperative use of ibuprofen 400 mg for a period of 48 hours, starting one hour before in-office bleaching treatment, does not reduce the absolute risk of tooth sensitivity but may reduce the tooth intensity up to one hour after the bleaching session without jeopardizing the whitening effect.

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REFERENCES

1. Leonard RFJR, Smith LR, Garland GE, Tiwana KK, Zaidel LA, Pugh GJR, & Lin NC (2007) Evaluation of side effects and patients perception during tooth bleaching *Journal of Esthetic Restorative Dentistry* **19(6)** 355-366.
2. Kihn PW (2007) Vital tooth whitening *Dental Clinics of North America* **51(2)** 319-331.
3. Marson FC, Sensi LG, Vieira LC, & Araújo E (2008) Clinical evaluation of in-office dental bleaching treatments with and without the use of light-activation sources *Operative Dentistry* **33(1)** 15-22.
4. Tay LY, Kose C, Loguercio AD, & Reis A (2009) Assessing the effect of a desensitizing agent used before in-office

tooth bleaching *Journal of the American Dental Association* **140(10)** 1245-1251.

5. Reis A, Dalanhol AP, Cunha TS, Kossatz S, & Loguercio AD (2011) Assessment of tooth sensitivity using a desensitizer before light-activated bleaching *Operative Dentistry* **36(1)** 12-17.
6. Kossatz S, Martins G, Loguercio AD, & Reis A (2012) Tooth sensitivity and bleaching effectiveness associated with use of a calcium-containing in-office bleaching gel *Journal of the American Dental Association* **143(12)** e81-e87.
7. Cooper JS, Bokmeyer TJ, & Bowles WH (1992) Penetration of the pulp chamber by carbamide peroxide bleaching agents *Journal of Endodontics* **18(7)** 315-317.
8. Costa CAS, Riehl H, Kina JF, Sacono NT, & Hebling J (2010) Human pulp responses to in-office tooth bleaching *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **109(4)** e59-e64.
9. Cook SP, & McCleskey EW (2002) Cell damage excites nociceptors through release of cytosolic ATP *Pain* **95(1-2)** 41-47.
10. Huynh MP, & Yagiela JA (2003) Current concepts in acute pain management *Journal of the California Dental Association* **31(5)** 419-427.
11. Leonard RH, Smith LR, Garland GE, & Caplan DJ (2004) Desensitizing agent efficacy during whitening in an at-risk population *Journal of Esthetic Restorative Dentistry* **16(1)** 49-56.
12. Armenio RV, Fitarelli F, Armenio MF, Demarco FF, Reis A, & Loguercio AD (2008) The effect of fluoride gel use on bleaching sensitivity: a double-blind randomized controlled clinical trial *Journal of the American Dental Association* **139(5)** 592-597.
13. Charakorn P, Cabanilla LL, Wagner WC, Foong WC, Shaheen J, Pregitzer R, & Schneider D (2009) The effect of preoperative ibuprofen on tooth sensitivity caused by in-office bleaching *Operative Dentistry* **34(2)** 131-135.
14. Suleyman H, Demican B, & Karagoz Y (2007) Anti-inflammatory and side effects of cyclooxygenase inhibitor. *Pharmacological Reports* **59(3)** 247-258.
15. Schulz KF, Altman DG, & Moher D (2010) CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials *British Medical Journal* **8(18)** 1-9.
16. Cardoso P, Reis A, Loguercio A, Vieira L, & Baratieri L (2010) Clinical effectiveness and tooth sensitivity associated with different bleaching times for a 10 percent carbamide peroxide *Journal of the American Dental Association* **141(10)** 1213-1220.
17. Jensen MP, Karoly P, Braver S. (1986) The measurement of clinical pain intensity: a comparison of six methods. *Pain* **27(1)** 117-26.
18. Caraceni A, Cherny N, Fainsinger R, Kaasa S, Poulain P, Radbruch L, De Conno F (2002) Pain measurement tools and methods in clinical research in palliative care: recommendations of an Expert Working Group of the European Association of Palliative Care. *Journal of Pain and Symptom Management* **23(3)** 239-55.
19. Noble B, Clark D, Meldrum M, ten Have H, Seymour J, Winslow M, Paz S (2005) The measurement of pain, 1945-2000. *Journal of Pain and Symptom Management* **29(1)** 14-21.
20. Lepinski AM, Hargreaves KM, Goodis HE, & Bowles WR (2000) Bradykinin levels in dental pulp by microdialysis *Journal of Endodontics* **26(12)** 744-747.
21. Nakanishi T, Shimizu H, Hosokawa Y, & Matsuo T (2001) An immunohistological study on cyclooxygenase-2 in human dental pulp *Journal of Endodontics* **27(6)** 385-388.
22. Holt CI, Hutchins MO, & Pileggi R (2005) A real time quantitative PCR analysis and correlation of COX-1 and COX-2 enzymes in inflamed dental pulps following administration of three different NSAIDs *Journal of Endodontics* **31(11)** 799-804.
23. Rodd H, & Boissonnade F (2000) Substance P expression in human tooth pulp in relation to caries and pain experience *European Journal of Oral Science* **108(6)** 467-474.
24. Greaves MW (1976) Anti-inflammatory action of corticosteroids *Postgrad Medical Journal* **52(612)** 631-633.
25. Chrousos GA, Kattah JC, Roy WB, Cleary PA, Optic Neuritcs Study Group (1993) Side effects of glucocorticoid treatment: experience of the Optic Neuritcs Treatment Trial *Journal of the American Medical Association* **269(16)** 2110-2112.
26. Närhi M, Jyväsjärvie E, Virtanen A, Huopaniemi T, Nqasswapa D, & Hirvonen T (1992) Role of intradental A- and C-type nerve fibres in dental pain mechanisms *Proceedings of the Finnish Dental Society* **88(Suppl 1)** 507-516.
27. Anderson DG, Chiego DJ, Glickman GN, & McCauley LK (1999) A clinical assessment of the effects of 10% carbamide peroxide gel on human pulp tissue *Journal of Endodontics* **25(4)** 247-250.
28. Imlay JA, & Linn S (1988) DNA damage and oxygen radical toxicity *Science* **3(4857)** 1302-1309.
29. Bellomo G (1991) Cell damage by oxygen free radicals *Cytotechnology* **5(1)** 71-73.
30. Haywood VB (2005) Treating sensitivity during tooth whitening *Compendium of Continuing Education in Dentistry* **26(9 Suppl 3)** 11-20.

4.2 Perioperative use of an anti-inflammatory drug on tooth sensitivity caused by in-office bleaching: a randomized, triple-blind clinical trial.

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ORIGINAL ARTICLE

Perioperative use of an anti-inflammatory drug on tooth sensitivity caused by in-office bleaching: a randomized, triple-blind clinical trial

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Abstract
Objectives The aim of this study was to determine the effect of etoricoxib 60 mg on tooth sensitivity (TS) caused by in-office bleaching.
Materials and methods A triple-blind, parallel design, randomized clinical trial was conducted on 30 healthy, young adults who received either a placebo or etoricoxib. The drugs were administered 1 h before the bleaching process and after 24 h. Treatment was performed with 35 % hydrogen peroxide gel. The TS was recorded on three scales: VAS, 0–4, and 0–100. Shade evaluations were performed before and 30 days after bleaching with a visual shade guide and a spectrophotometer. The percentage of patients who reported TS at least once during treatment and the TS intensity were evaluated by Fisher's exact and Mann–Whitney *U* tests, respectively. Tooth color changes were evaluated by repeated measures ANOVA.
Results There were no significant differences in the percentage of patients with TS, intensity of TS, and color between the groups.
Conclusions and clinical significance The anti-inflammatory medication etoricoxib 60 mg was unable to reduce the presence and intensity of TS. NCT01300780 (protocol No. 17838/2010).

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Keywords Tooth bleaching · Randomized controlled clinical trial · Tooth sensitivity · Anti-inflammatory agents

Introduction

Vital tooth bleaching with a peroxide gel is generally recognized as a safe and effective procedure [1] and can be performed either using the at-home and in-office bleaching protocol [2]. Even though the at-home bleaching system is the most frequently recommended treatment for vital teeth, some patients do not want to use a bleaching tray or do not want to wait 2 to 3 weeks to see the results of the treatment [3]. Thus, another bleaching option is the in-office bleaching procedure. The effectiveness of this bleaching procedure is well established in the literature with an overall color change of 5 to 8 shade guide units (SGU) after two in-office bleaching sessions [2–9].

However, with the in-office procedure using 35 % hydrogen peroxide, there is a long history of tooth sensitivity (TS) and gingival irritation [10, 11]. Incidence levels of TS have been reported to be as high as 87 % [3, 5, 6], and this has been considered the main reason why patients have not successfully completed their whitening treatment [3, 8]. For instance, Schulte et al. [12] reported that of 28 subjects submitted to at-home bleaching, 4 discontinued the bleaching protocol because of TS.

As reported by Markowitz [13], many authors claim that bleaching-related pain is the result of the hydrodynamic theory of dentin sensitivity [14, 15]. However, although pain in bleached teeth can be evoked by thermal or other stimuli, as in dentin hypersensitivity, most patients complain of tingling or shooting pain (zingers) [15] without provoking stimuli. Moreover, pain after bleaching can affect intact

teeth without exposed dentin, which is in sharp contrast to dentin sensitivity when pain occurs in teeth with exposed dentin [13]. Based on this, it is suggested that TS resulting from bleaching may be due to the easy passage of the peroxide through the enamel and dentin and the production of pulp inflammation [16].

The observation that certain bleaching procedures increase pulpal expression of substance-P (a nerve-released vasoactive peptide) suggests that neurogenic inflammation may play a role in the onset of this side effect [17]; however, this should be further investigated. In addition to substance-P, arachidonic acid metabolites, such as prostaglandins (PGs), have a recognized role in triggering and subsequently potentiating nociceptive impulses that are transmitted to the central nervous system for the perception of pain [18]. The generation of PGs, which have been known to play a critical role in the pathogenesis of pulpal disease involves the cyclo-oxygenase (COX) pathway. There are at least two variants of COX: the COX-1, which is involved in physiological functions, and inducible COX-2, which is believed to be involved in the inflammatory response [19] and has been considered responsible for the production of PGs mediating inflammation and pain in inflamed pulps [20].

There are a number of materials and techniques for reducing TS that arises from bleaching [21]. The use of desensitizing agents, such as fluorides and potassium nitrate before or after at-home [22, 23] and in-office bleaching therapies [3, 5] or their inclusion in the bleaching formulations [22, 24] has been shown to be capable of reducing the experience of TS during bleaching treatment.

Another approach recently investigated was the preoperative use of ibuprofen [25]. Although this clinical alternative seems to be promising, the preoperative use of a single dose of ibuprofen 600 mg before the in-office bleaching protocol was shown to reduce TS only during but not after the treatment period. Ibuprofen is a nonsteroidal anti-inflammatory drug, which is believed to work through the inhibition of both constitutive COX-1 and inducible COX-2 [19].

Perhaps the use of a more selective anti-inflammatory drug, capable of inhibiting only the inducible COX-2 enzyme may be more effective in preventing the TS caused by the inflammatory response produced by in-office bleaching. In addition, the drug posology of the selected anti-inflammatory drug is more patient friendly since it requires taking the medicine once every 24 h. To the extent of the authors' knowledge, no study has so far addressed the use of a selective anti-inflammatory drug on TS. Therefore the aim of the present investigation was to determine the effect of etoricoxib 60 mg on TS caused by in-office bleaching. In the present study, we tested the hypothesis that the preventive use of a selective COX-2 anti-inflammatory drug would not affect the absolute risk of TS.

Materials and methods

This clinical investigation was approved (protocol No. 17836/2010) by the scientific review committee and by the committee for the protection of human subjects of the local university. The experimental design was in accordance with the CONSORT statement [26]. Based on pre-established criteria, 30 volunteers from the city of Guarapuava, Paraná, Brazil were selected for this study. Two weeks before the bleaching procedures, all the volunteers underwent dental screening and dental prophylaxis with pumice and water in a rubber cup and signed a term of free and informed consent.

Study design This was a randomized, triple-blind, placebo-controlled, parallel-group clinical trial, with an equal allocation rate to receive either one of two treatments. The study took place in the clinic of the Brazilian Association of Dentistry in Guarapuava, Paraná from August 2010 to December 2010.

Inclusion and exclusion criteria Patients included in this clinical trial were at least 18 years old and had good general and oral health. Participants were recruited by means of radio and TV advertisement. A total of 247 participants were examined in a dental chair to check whether they met the inclusion and exclusion criteria (Fig. 1). The participants were required to have six caries-free maxillary anterior teeth, without restorations on the labial surfaces. The central incisors had to be shade C2 or darker as judged by comparison with a value-oriented shade guide (Vita Lumin, Vita Zahnfabrik, Bad Säckingen, Germany). Participants who had undergone tooth-whitening procedures, presented anterior restorations, were pregnant/lactating, had severe internal tooth discoloration (tetracycline stains, fluorosis, and pulpless teeth), were taking any type of medicine, had bruxism habits, or any other pathology that could cause sensitivity (such as recession and dentin exposure) were excluded from the study, since they would not be immediately eligible for a cosmetic treatment, such as bleaching. Participants who reported some earlier or present health problems in the stomach, heart, kidney and liver, or participants continuously using any with anti-inflammatory and antioxidant action were excluded from the study.

Sample size calculation The primary outcome of this study was the absolute risk of TS. The absolute risk of TS was reported to be approximately 86 % [3] for the bleaching product Whiteness HP Maxx (FGM, Joinville, SC, Brazil). Thus, 30 patients were required to have an 80 % chance of detecting, as significant at the two-sided 5 % level, a decrease in the primary outcome measure from 86 % in the control group to 41 % in the experimental group.

Study intervention Participants were randomly stratified by sex into the placebo and etoricoxib groups. The randomization

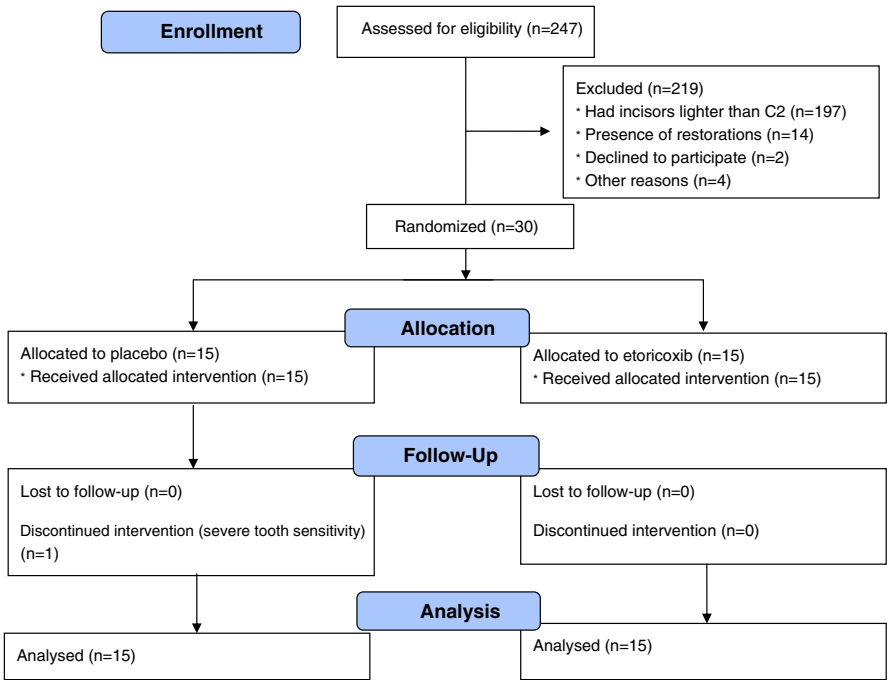

Fig. 1 Flow diagram of the clinical trial including detailed information on the excluded participants

process was performed by computer-generated tables by a third person not involved in the research protocol. Details of the allocated groups were recorded on cards contained in sequentially numbered, opaque, sealed envelopes. These were prepared by a third person not involved in any of the phases of the clinical trial. Once the participant was eligible for the procedure, and completed all baseline assessments, the allocation assignment was revealed by this envelope being opened by the mentioned third person. Neither the participant nor the operator knew the group allocation, both being blinded to the protocol.

The participants from the placebo group received a placebo (Talco pharma SM-200-Henrifarma produtos químicos e farmacêuticos LTDA, São Paulo, SP, Brazil) and participants from the etoricoxib group received a dose of a selective COX-2 inhibitor etoricoxib 60 mg (Arcoxia, MSD, Campinas, SP, Brazil). All the participants were watched to ensure that they took the drugs or placebo 1 h before treatment. The drugs were similar in appearance. A second dose of placebo or etoricoxib (60 mg) was administered 24 h after the first dose. At the time the participants were required to take the second dose of the medicine, the research auxiliary called him/her and asked him/her to take the medicine. This procedure was implemented to increase adherence to the protocol.

The gingival tissue of the teeth to be bleached was isolated using a light-polymerized resin dam (Top Dam, FGM, Joinville, SC, Brazil). The 35 % hydrogen peroxide gel (Whiteness HP Maxx, FGM) was used in three 15-min applications for both groups in accordance with the manufacturer's directions. The in-office bleaching agent was refreshed every 15 min during the 45-min application period. Two bleaching sessions with 1 week interval between them were performed. All participants were instructed to brush their teeth regularly using fluoridated toothpaste (Sorriso Fresh, Colgate-Palmolive, São Paulo, SP, Brazil).

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

For the subjective examination, the 16 shade guide tabs were arranged from highest (B1) to lowest (C4) value, so that the color C2 was set at No. 7. Although this scale is not linear in the truest sense, the changes were treated as though they represented a continuous and approximately linear ranking for the purpose of analysis [5, 6, 11]. The measurement area of interest for shade matching was the middle one third of the facial surface of the anterior central incisor [11]. For calibration purposes, five patients who were not included in the sample because they were used in the pilot study, participated in the training phase of this study. The two examiners, blinded to the allocation assignment, scheduled these patients 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.

For the objective evaluation, a preliminary impression of the maxillary arch was made using dense silicone Adsil (Vigodent S/A Indústria e Comércio, Rio de Janeiro, RJ, Brazil). The impression was extended to the maxillary canine and served as a standard color 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 metal device with well-formed borders, with a radius of 3 mm [11]. Only one operator took all the measurements in all 30 patients, using Vita Easyshade (Easyshade, Vident, Brea, CA) before and 30 days after the bleaching therapy. The shade was determined using the parameters of the Easyshade device where the following values were indicated: 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 color comparison before and after treatment is given by the difference between the two colors (ΔE), which is calculated using the formula: $\Delta E=((\Delta L^*)2+(\Delta a^*)2+(\Delta b^*)\ 2)1/2$ [11].

TS evaluation The patients recorded their perception of TS during the first and second bleaching sessions using three pain scales. A 5-point verbal rating scale (0=none, 1=mild, 2=moderate, 3=considerable, and 4=severe] [3, 5], a 0 to 100 numerical rating scale [27] and a visual analogue scale [25–29] using a 10-cm horizontal line with words "no pain" at one end and "worst pain" at the opposite end were used in this study. Subjects were asked to record whether they experienced TS during the treatment up to 1 h after, from 1 to 24 h and from 24 to 48 h after bleaching. The worst scores/numerical values obtained in both bleaching sessions were considered for statistical purposes. The values were arranged into two categories: percentage of patients that reported TS at least once during treatment (absolute risk of TS) and overall TS intensity.

Statistical analysis The analysis was performed after the intention-to-treat protocol and involved all participants who were randomly assigned [26]. The statistician was blinded to the study groups. The agreement between the examiners' objective evaluation was evaluated using the kappa statistic. The primary outcome absolute risk of TS was compared by using the Fisher's exact test at a 5 % level of significance. The relative risk as well as the confidence interval for the effect size was calculated.

TS intensity (secondary outcome) was also statistically analyzed. The mean/median and standard deviation/interquartile range of the three pain scales were calculated. Color change, another secondary endpoint, was used to assess the efficacy of the bleaching treatment. For subjective evaluation, the means and standard deviations of SGU at baseline and 30 days after bleaching for each group were calculated. In order to evaluate whether the bleaching therapies were effective or not, the data from SGU of both groups were submitted to a two-way repeated measures ANOVA. A post-hoc analysis (Tukey's test) was used to make pairwise comparisons. The ΔL, Δa, Δb, and ΔE values were evaluated by Student's t test.

The data sets were plotted on histograms and inspected for normal distributions. Some data did not appear to be normally distributed, and therefore, nonparametric statistical tests were used to compare the various treatments. Statistical analyses of three pain scales comparing the two groups at the three different assessment points were 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 set at $\alpha=0.05$. p values less than or equal to 5 % indicated significant differences.

Table 1 Comparison of the number of patients who experienced tooth sensitivity during the bleaching regimen in both groups along with absolute and relative risks

Treatment	Tooth sensitivity (number of participants)		Absolute risk (95 % CI)	Relative risk (95 % CI)
	Yes	No		
Placebo	9	6	60 (35–80)	1.2 (0.73–2.0)
Etoricoxib	11	4	73 (48–89)	

Fisher's test ($p=0.69$)

Results

The mean age (years) of the participants in this study was similar between the groups (placebo, 26.4±6.8 and etoricoxib, 26.7±6.1). Fifty three and sixty percent of the participants from the placebo and etoricoxib groups were men. Figure 1

Table 2 Comparison of the number of patients (in percent) who experienced tooth sensitivity at the different assessment points for the two treatment groups

	Placebo	Etoricoxib
Up to 1 h	8 (53) Aa	9 (60) Aa
1 to 24 h	9 (60) Ab	11 (73) Ab
24 to 48 h	0 (0) Bc	2 (13) Bc

Within columns, similar uppercase letters indicate similar percentages. Within rows, similar lowercase letters indicate similar percentages (Chi-square test, $\alpha=0.05$)

Table 3 Comparison of tooth sensitivity intensity experienced by patients from the treatment groups at different assessment points using three pain scales

	0–4[a]		0–10[b]		0–100[b]	
	Placebo	Etoricoxib	Placebo	Etoricoxib	Placebo	Etoricoxib
Up to 1 h	2 (0/4) a, A	1 (0/3) a, A	3.3±2.9 a, A	2.6±2.4 a, A	40.1±28.7 a, A	37.3±26.7 a, A
1 to 24 h	1 (0/4) a, A	1 (0/3) a, A	2.4±2.7 a, A	2.2±2.8 a, A	30.6±28.6 a, A	22.5±29.3 a, A
24 to 48 h	0 (0/0) b, B	0 (0/1) b, B	0±0 b, B	0.1±0.4 b, B	0±0 b, B	1.6±4.5 b, B

Comparisons are valid only within the same pain scale. At each period, the two treatments were compared with Mann–Whitney *U* test and differences are represented by different lowercase letters. For each treatment, the three periods were compared with the Friedman test (α=0.05) and differences are represented by different uppercase letters

[a] Median (minimum/maximum) values

[b] Means and standard deviations

depicts the participant flow diagram in the different phases of the study design.

Tooth sensitivity

The data from 30 patients were used in this study after the intention-to-treat analysis. One patient from the placebo group received an analgesic after the second bleaching session due to considerable TS. In regard to the absolute risk of TS (primary outcome), no significant difference was observed between groups, as shown in Table 1 (p=0.69). The relative risk together with the 95 % confidence interval also shows evidence that the use of the experimental drug had no effect on the reduction of TS.

Table 2 depicts the number of participants who experienced TS during 48 h after bleaching. Most of the TS complaints occurred within the first 24 h (p<0.001), and only one of the participants experienced pain after 24 h. In regard to TS intensity (Table 3), the groups did not differ statistically according to the three pain scales used in this study (p>0.05).

Color evaluation

Significant whitening was observed in both study groups by means of the subjective and objective evaluation methods (p< 0.001). Whitening of approximately 4 to 5 SGU was detected for both groups (Table 4) and the ΔE varied from 7.2 to 8.1 (Table 4). The results of the subjective (visual shade guide) and the objective evaluation (spectrophotometer) matched the hypothesis of equality between the values after bleaching (p≈0.6 for both methods).

Discussion

Three pain scales were used to investigate the intensity of TS in this study, in order to allow comparison with previous literature studies that used different pain scales. The results of the present study show that the scales provided similar ratings, which is in agreement with previous literature findings on this topic [27]. Although this was not the main focus of the present investigation, it could also be observed that the experience of TS for most of the participants occurred within the first 24 h after bleaching, adding further evidence to the fact that this TS is transient.

As mentioned in the introduction, tooth whitening has been shown to produce a moderate inflammation process in the pulp, with visible changes in odontoblastic layer [30], however, contrary to our previous expectation the use of a selective anti-inflammatory drug (etoricoxib 60 mg) in a preventive approach was incapable of reducing TS arising from bleaching. One could attribute this to a possible lack of COX-2 expression in the pulp of bleached teeth soon after the bleaching procedure. Although an earlier study [20]

Table 4 Means and standard deviations of shade guide units (Vita Classical shade guide) and ΔL, Δa, Δb and ΔE (spectrophotometer) at different assessment points for the two treatment groups

	Placebo	Etoricoxib
Subjective evaluation—visual shade guide		
Time assessment		
Baseline	9.3±2.0 A	8.3±1.4 A
30 days after bleaching	3.9±1.8 B	4.5±1.6 B
Objective evaluation—spectrophotometer		
CIELab parameter		
ΔL	1.2±2.2 A	2.3±1.8 A
Δa	−0.73±1.0 B	−0.99±1.2 B
Δb	−6.7±2.3 C	−7.3±3.4 C
ΔE	7.2±2.5 D	8.1±3.3 D

Means indicated by the same uppercase letters indicate statistically similar means (two-way ANOVA, α=0.05). Comparisons are only valid within rows. Similar upper case letters indicate statistically similar means (Student's *t* test, α=0.05)

identified the presence of COX-2 expressing cells in inflamed human pulps by the immunohistological method, the inflamed pulp was obtained from carious teeth, and since caries disease progression is slow [31], it does not seem to trigger an acute inflammatory response such as bleaching probably does [16, 21, 30]. Thus, the presence of COX-2 in the pulp of carious teeth does not necessarily mean that the inflamed pulps of bleached teeth will express this enzyme immediately after bleaching.

Another explanation for the lack of efficacy of etoricoxib in preventing TS is that several other inflammatory mediators [32] are likely to be involved in the inflammatory reaction in the pulp tissue, which leads to pain and symptoms of neurogenic inflammation. For instance, bradykinin [33] and substance-P have long been known to be involved in the process of pulp pain and inflammation [17, 34]. Unfortunately, etoricoxib cannot prevent the production of these mediators. If many mediators are synergistically acting to produce both pain and inflammatory reaction after bleaching, an anti-inflammatory agent that inhibits several initial inflammatory events, such as the glucocorticoids [35], may be more effective to reduce the TS arising from bleaching. Glucocorticoids are known to inhibit the expression of multiple inflammatory genes (cytokines, enzymes, receptors, and adhesion molecules) and inhibit the transcription of several cytokines that are relevant in inflammatory diseases [36]. This should be the focus of future clinical investigations that attempt to reduce bleaching-related TS.

It is known that dental pulp is densely innervated with sensory afferents with conduction velocities in the Aβ, Aδ, and C-fiber range [37]. One may hypothesize that hydrogen peroxide may cause direct cellular damage to nerve cells via free radicals and other reactive oxygen species [38] triggering nerve fiber depolarization. In fact, hydrogen peroxide can, under certain conditions, react with a variety of cellular components, which include lipid peroxidation of membrane protein and nucleic acid oxidation [39, 40]. If this is the cause of TS, the use of an anti-inflammatory drug, such the one investigated in the present study, would not reduce the pain experience. Other alternative approaches such as the use of anti-oxidant drugs, not yet investigated, and the use of desensitizers [3] would be more effective. The latter cannot reduce the cell damage, but it can reduce the excitability of the intradental nerves [41, 42]. A potassium nitrate agent used before bleaching was shown to reduce the experience of TS [3] since it penetrates through the enamel to travel to the dentin-pulp complex where it creates a calming effect on the nerve by reducing the transmission of nerve impulses [41, 42].

The efficacy of any drug is dependent on the selected dose. Thus, one cannot rule out the fact that a low dose could be the reason for the lack of efficacy of the drug in the present investigation. An earlier study evaluating the effect of different doses of etoricoxib on the acute pain resulting from dental surgery reported lower pain with the use of higher doses [43].

In regard to the bleaching outcome, the results of this study indicated that both groups demonstrated equivalent and significant tooth color enhancement when compared with baseline (Table 1). It is difficult to compare color change after in-office bleaching with existing literature due to the different methods of measurement (shade guides and spectrophotometers) and different units of measurement (CIELab system, SGU, etc.) used. However studies that used 35 % hydrogen peroxide and reported their results in SGU usually observed an overall color change of 5 to 8 SGU after two bleaching sessions [3–9], which is in agreement with the results of the present investigation.

Finally, one could not omit mentioning that the small sample size is a clear limitation of the present study. The study was designed to find a high effect size, i.e., reduction of approximately 50 % in the absolute risk of TS between participants from the placebo and the experimental group. Thus, we can conclude that an effect size as large as this was not observed with a power of 80 %, but we cannot rule out the fact that smaller effect sizes do exist. Use of the same experimental design to conduct studies with larger sample sizes should be encouraged to rule out this hypothesis. Moreover, the selected sample was mainly composed of young participants, which also limits the ability to generalize our findings to the general population of older adults.

Conclusions

Within the limitations of our study, we conclude that the use of etoricoxib 60 mg does not reduce the experience and intensity of TS.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Giniger M, MacDonald J, Felix H (2005) The clinical performance of professionally dispensed bleaching with added amorphous calcium phosphate. J Am Dent Assoc 136:383–392

2. Bernardon JK, Sartori N, Ballarin A, Perdigão J, Lopes GC, Baratieri LN (2010) Clinical performance of vital bleaching techniques. Oper Dent 35:3–10
3. Tay LY, Kose C, Loguercio AD, Reis A (2009) Assessing the effect of a desensitizing agent used before in-office tooth bleaching. J Am Dent Assoc 140:1245–1251
4. Strobl A, Gutknecht N, Franzen R, Hilgers RD, Lampert F, Meister J (2010) Laser-assisted in-office bleaching using a neodymium:yttrium-aluminum-garnet laser: an in vivo study. Lasers Med Sci 25:503–509
5. Reis A, Dalanhol AP, Cunha TS, Kossatz S, Loguercio AD (2011) Assessment of tooth sensitivity using a desensitizer before light-activated bleaching. Oper Dent 36:12–17
6. Kossatz S, Martins G, Loguercio AD, Reis A (2012) Tooth sensitivity and bleaching effectiveness associated with use of a calcium-containing in-office bleaching gel. J Am Dent Assoc 143:e81–e87
7. Tay LY, Kose C, Herrera DR, Reis A, Loguercio AD (2012) Long-term efficacy of in-office and at-home bleaching: a 2-year double-blind randomized clinical trial. Am J Dent 25:199–204
8. Basting R, Amaral F, França F, Flório F (2012) Clinical comparative study of the effectiveness of and tooth sensitivity to 10 % and 20 % carbamide peroxide home-use and 35 % and 38 % hydrogen peroxide in-office bleaching materials containing desensitizing agents. Oper Dent 37:464–473
9. Almeida LC, Riehl H, Santos PH, Sundfeld ML, Briso AL (2012) Clinical evaluation of the effectiveness of different bleaching therapies in vital teeth. Int J Periodontics Restorative Dent 32:303–309
10. Kihn PW (2007) Vital tooth whitening. Dent Clin North Am 51:319–331
11. Marson FC, Sensi LG, Vieira LC, Araújo E (2008) Clinical evaluation of in-office dental bleaching treatments with and without the use of light-activation sources. Oper Dent 33:15–22
12. Schulte JR, Morrissete DB, Gasior EJ, Czajewski MV (1994) The effect of bleaching application time on the dental pulp. J Am Dent Assoc 125:1330–1335
13. Markowitz K (2010) Pretty painful: why does tooth bleaching hurt? Med Hypotheses 74:835–840
14. Swift EJ Jr (2005) Tooth sensitivity and whitening. Compend Contin Educ Dent 26:4–10
15. Haywood VB (2005) Treating sensitivity during tooth whitening. Compend Contin Educ Dent 26:11–20
16. Cooper JS, Bokmeyer TJ, Bowles WH (1992) Penetration of the pulp chamber by carbamide peroxide bleaching agents. J Endod 18:315–317
17. Caviedes-Bucheli J, Ariza-Garcia G, Restrepo-Mendez S, Ríos-Osorio N, Lombana N, Muñoz HR (2008) The effect of tooth bleaching on substance P expression in human dental pulp. J Endod 34:1462–1465
18. Huynh MP, Yagiela JA (2003) Current concepts in acute pain management. J Calif Dent Assoc 31:419–427
19. Suleyman H, Demircan B, Karagoz Y (2007) Anti-inflammatory and side effects of cyclooxygenase inhibitor. Pharmacol Rep 59:247–258
20. Nakanishi T, Shimizu H, Hosokawa Y, Matsuo T (2001) An immunohistological study on cyclooxygenase-2 in human dental pulp. J Endod 27:385–388
21. Haywood VB (2005) Treating sensitivity during tooth whitening. Compend Contin Educ Dent 26:11–20
22. Leonard RH, Smith LR, Garland GE, Caplan DJ (2004) Desensitizing agent efficacy during whitening in an at-risk population. J Esthet Restor Dent 16:49–56
23. Armenio RV, Fitarelli F, Armenio MF, Demarco FF, Reis A, Loguercio AD (2008) The effect of fluoride gel use on bleaching sensitivity: a double-blind randomized controlled clinical trial. J Am Dent Assoc 139:592–597
24. Jorgensen MG, Carroll WB (2002) Incidence of tooth sensitivity after home whitening treatment. J Am Dent Assoc 133:1076–1082
25. Charakorn P, Cabanilla LL, Wagner WC, Foong WC, Shaheen J, Pregitzer R, Schneider D (2009) The effect of preoperative ibuprofen on tooth sensitivity caused by in-office bleaching. Oper Dent 34:131–135
26. Schulz KF, Altman DG, Moher D (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomized trials. Brit Med J 8:1–9
27. Jensen MP, Karoly P, Braver S (1986) The measurement of clinical pain intensity: a comparison of six methods. Pain 27:117–126
28. Caraceni A, Cherny N, Fainsinger R, Kaasa S, Poulain P, Radbruch L, De Conno F (2002) Pain measurement tools and methods in clinical research in palliative care: recommendations of an expert working group of the European association of palliative care. J Pain Sym Man 23:239–255
29. Noble B, Clark D, Meldrum M, ten Have H, Seymour J, Winslow M, Paz S (2005) The measurement of pain. J Pain Sym Man 29:1945–2000
30. Costa CAS, Riehl H, Kina JF, Sacono NT, Hebling J (2010) Human pulp responses to in-office tooth bleaching. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 109:e59–e64
31. Anusavice K (2001) Clinical decision-making for coronal caries management in the permanent dentition. J Dent Edu 65:1143–1146
32. Holt CI, Hutchins MO, Pileggi R (2005) A real time quantitative PCR analysis and correlation of COX-1 and COX-2 enzymes in inflamed dental pulps following administration of three different NSAIDs. J Endod 31:799–804
33. Lepinski AM, Hargreaves KM, Goodis HE, Bowles WR (2000) Bradykinin levels in dental pulp by microdialysis. J Endod 26:744–747
34. Rodd H, Boissonnade F (2000) Substance P expression in human tooth pulp in relation to caries and pain experience. Eur J Oral Sci 108:467–474
35. Greaves MW (1976) Anti-inflammatory action of corticosteroids. Postgrad Med J 52:631–33
36. Barnes PJ (1998) Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin Sci 94:557–572
37. Närhi M, Jyväsjärvi E, Virtanen A, Huopaniemi T, Ngassapa D, Hirvonen T (1992) Role of intradental A- and C-type nerve fibres in dental pain mechanisms. Proc Finn Dent Soc 88:507–516
38. Anderson DG, Chiego DJ, Glickman GN, McCauley LK (1999) A clinical assessment of the effects of 10 % carbamide peroxide gel on human pulp tissue. J Endod 25:247–250
39. Imlay JA, Linn S (1988) DNA damage and oxygen radical toxicity. Science 3:1302–1309
40. Bellomo G (1991) Cell damage by oxygen free radicals. Cytotechnology 5:71–73
41. Kim S (1986) Hypersensitive teeth: desensitization of pulpal sensory nerves. J Endod 12:482–485
42. Markowitz K, Bilotto G, Kim S (1991) Decreasing intradental nerve activity in the cat with potassium and divalent cations. Arch Oral Biol 36:1–7
43. Malmstron K, Sapre A, Coughlin H, Agrawal NG, Mazenko RS, Fricke JR Jr (2004) Etoricoxib in acute pain associated with dental surgery: a randomized, double-blind, placebo- and active comparator-controlled dose-ranging study. Clin Ther 26:667–679

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

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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 bleaching-induced tooth sensitivity experience.

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 similar risk of tooth sensitivity ($p>0.05$). The perioperative use of an antioxidant, such as ascorbic acid (500 mg, three times daily) peroral route, was not able to avoid 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, and they excite and sensitize pulp nociceptors,[14] leading to the transmission of the pain stimuli.

In an attempt to reduce the damage produced by HP on 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 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 is 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 by reduction of the risk of TS and its intensity. However, the only available method to deliver an antioxidant to the pulp tissue is peroral route or intravenously. Therefore, this study attempted to investigate if the perioperative use of an antioxidant peroral 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 should have had at least six maxillary and mandibular anterior teeth that were caries-free and without restorations on the labial surfaces. Selected participants should have had central incisors C2 or darker as judged by comparison with a value-oriented shade guide (Vita Lumin, Vita Zahnfabrik, Bad Säckingen, Germany).

Participants who had 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, dentine exposure) were excluded. This procedure was done to minimize confounding experimental variables or side effects from bleaching. Participants who reported a history or presented

health problems in 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 by 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 auxiliary 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 pill of placebo (Talco pharma S M-200 Henrifarma, São Paulo, SP, Brazil), and participants from the experimental group received a dose of 500 mg 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 during a period of 48 hours. Participants were reminded by research auxiliary 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 report pain up to 48 hours.[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: the 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 one third of the facial surface of the anterior central incisor. This measurement was done at baseline and 30 days after bleaching allowing 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, they would reach 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, 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%.

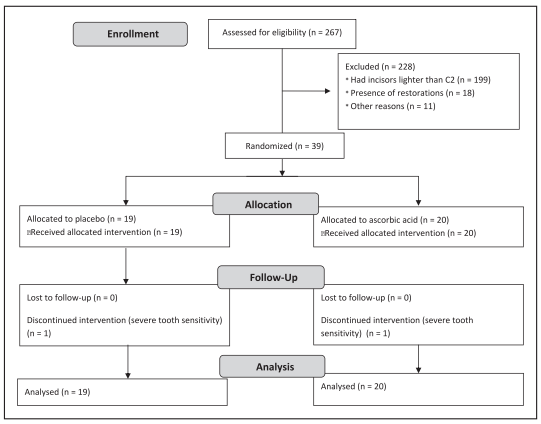

Figure 1. *Flow diagram of the clinical trial including detailed information on the excluded participants.*

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 analgesic after the first bleaching session, and one

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).

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, the ascorbic acid, also known as vitamin C, is the most popular sold, and gram doses are promoted for preventing and treating the common cold, managing stress, and enhancing well-being. Ascorbic acid is an electron

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).*

donor, and because of this, is a potent water-soluble antioxidant in humans.[36] In addition, it 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 one's previous expectation, the perioperative use of ascorbic acid was not capable of reducing 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 on the extracellular fluid after oral administration of 500 mg of ascorbic acid. Under peroral route 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 showed 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 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 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 enamel surface, as a way to allow fast delivery of the antioxidant on the pulp tissue; however, it is probable that the ascorbic acid will not penetrate in 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 as compared with 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.

The 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. Besides that, 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, one should mention that 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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REFERENCES

1. Leonard RH Jr, Smith LR, Garland GE, Tiwana KK, Zaidel LA, Pugh G Jr, & Lin NC (2007) Evaluation of side effects and patients' perceptions during tooth bleaching *Journal of Esthetic and Restorative Dentistry* **19(6)** 355-364.
2. Hattab FN, Qudeimat MA, & al-Rimawi HS (1999) Dental discoloration: An overview *Journal of Esthetic Dentistry* **11(6)** 291-310.
3. Perdigao J, Baratieri LN, & Arcari GM (2004) Contemporary trends and techniques in tooth whitening: A review *Practical Procedures & Aesthetic Dentistry* **16(3)** 185-192; quiz 194.
4. Kihn PW (2007) Vital tooth whitening *Dental Clinics of North America* **51(2)** 319-331, viii.
5. Marson FC, Sensi LG, Vieira LC, & Araujo E (2008) Clinical evaluation of in-office dental bleaching treatments with and without the use of light-activation sources *Operative Dentistry* **33(1)** 15-22.
6. Reis A, Dalanhol AP, Cunha TS, Kossatz S, & Loguercio AD (2011) Assessment of tooth sensitivity using a desensitizer before light-activated bleaching *Operative Dentistry* **36(1)** 12-17.
7. Haywood VB (2005) Treating sensitivity during tooth whitening *Compendium of Continuing Education in Dentistry* **269(Supplement 3)** 11-20.
8. Tay LY, Kose C, Loguercio AD, & Reis A (2009) Assessing the effect of a desensitizing agent used before in-office tooth bleaching *Journal of the American Dental Association* **140(10)** 1245-1251.
9. Cooper JS, Bokmeyer TJ, & Bowles WH (1992) Penetration of the pulp chamber by carbamide peroxide bleaching agents *Journal of Endodontics* **18(7)** 315-317.
10. Costa CA, Riehl H, Kina JF, Sacono NT, & Hebling J (2010) Human pulp responses to in-office tooth bleaching *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **109(4)** e59-64.
11. Kanno S, Ishikawa M, Takayanagi M, Takayanagi Y, & Sasaki K (2000) Characterization of hydrogen peroxide-induced apoptosis in mouse primary cultured hepatocytes *Biological and Pharmaceutical Bulletin* **23(1)** 37-42.
12. Kanno S, Shouji A, Asou K, & Ishikawa M (2003) Effects of naringin on hydrogen peroxide-induced cytotoxicity and apoptosis in P388 cells *Journal of Pharmacological Sciences* **92(2)** 166-170.
13. Cook SP, & McCleskey EW (2002) Cell damage excites nociceptors through release of cytosolic ATP *Pain* **95(1-2)** 41-47.
14. Huynh MP, & Yagiela JA (2003) Current concepts in acute pain management *Journal of the California Dental Association* **31(5)** 419-427.
15. Lima AF, Lessa FC, Mancini MN, Hebling J, Costa CA, & Marchi GM (2010) Transdentinal protective role of sodium ascorbate against the cytopathic effects of H_2O_2 released from bleaching agents *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **109(4)** e70-76.
16. Freire A, Souza EM, de Menezes Caldas DB, Rosa EA, Bordin CF, de Carvalho RM, & Vieira S (2009) Reaction kinetics of sodium ascorbate and dental bleaching gel *Journal of Dentistry* **37(12)** 932-936.
17. Schulz KF, Altman DG, & Moher D (2010) CONSORT 2010 Statement: Updated guidelines for reporting parallel group randomised trials *BMC Medicine* **8** 18.
18. Kossatz S, Dalanhol AP, Cunha T, Loguercio A, & Reis A (2011) Effect of light activation on tooth sensitivity after in-office bleaching *Operative Dentistry* **36(3)** 251-257.
19. Padayatty SJ, Sun H, Wang Y, Riordan HD, Hewitt SM, Katz A, Wesley RA, & Levine M (2004) Vitamin C pharmacokinetics: Implications for oral and intravenous use *Annals of Internal Medicine* **140(7)** 533-537.
20. Gallagher A, Maggio B, Bowman J, Borden L, Mason S, & Felix H (2002) Clinical study to compare two in-office (chairside) whitening systems *Journal of Clinical Dentistry* **13(6)** 219-224.
21. Al Shethri S, Matis BA, Cochran MA, Zekonis R, & Stropes M (2003) A clinical evaluation of two in-office bleaching products *Operative Dentistry* **28(5)** 488-495.
22. Zekonis R, Matis BA, Cochran MA, Al Shetri SE, Eckert GJ, & Carlson TJ (2003) Clinical evaluation of in-office and at-home bleaching treatments *Operative Dentistry* **28(2)** 114-121.
23. Jensen MP, Karoly P, & Braver S (1986) The measurement of clinical pain intensity: A comparison of six methods *Pain* **27(1)** 117-126.

24. Caraceni A, Cherny N, Fainsinger R, Kaasa S, Poulain P, Radbruch L, & De Conno F (2002) Pain measurement tools and methods in clinical research in palliative care: Recommendations of an Expert Working Group of the European Association of Palliative Care *Journal of Pain and Symptom Management* **23(3)** 239-255.

25. Noble B, Clark D, Meldrum M, ten Have H, Seymour J, Winslow M, & Paz S (2005) The measurement of pain, 1945-2000 *Journal of Pain and Symptom Management* **29(1)** 14-21.

26. Charakorn P, Cabanilla LL, Wagner WC, Foong WC, Shaheen J, Pregitzer R, & Schneider D (2009) The effect of preoperative ibuprofen on tooth sensitivity caused by in-office bleaching *Operative Dentistry* **34(2)** 131-135.

27. Kugel G, Ferreira S, Sharma S, Barker ML, & Gerlach RW (2009) Clinical trial assessing light enhancement of in-office tooth whitening *Journal of Esthetic and Restorative Dentistry* **21(5)** 336-347.

28. Tang B, & Millar BJ (2010) Effect of chewing gum on tooth sensitivity following whitening *British Dental Journal* **208(12)** 571-577.

29. Reis A, Tay LY, Herrera DR, Kossatz S, & Loguercio AD (2011) Clinical effects of prolonged application time of an in-office bleaching gel *Operative Dentistry* **36(6)** 590-596.

30. Shackelford RE, Kaufmann WK, & Paules RS (2000) Oxidative stress and cell cycle checkpoint function *Free Radical Biology & Medicine* **28(9)** 1387-1404.

31. Martindale JL, & Holbrook NJ (2002) Cellular response to oxidative stress: Signaling for suicide and survival *Journal of Cellular Physiology* **192(1)** 1-15.

32. Halliwell B, & Cross CE (1994) Oxygen-derived species: Their relation to human disease and environmental stress *Environmental Health Perspectives* **102(Supplement 10)** 5-12.

33. de Lima AF, Lessa FC, Gasparoto Mancini MN, Hebling J, de Souza Costa CA, & Marchi GM (2009) Cytotoxic effects of different concentrations of a carbamide peroxide bleaching gel on odontoblast-like cells MDPC-23 *Journal of Biomedical Materials Research. Part B, Applied Biomaterials* **90(2)** 907-912.

34. Hanks CT, Fat JC, Wataha JC, & Corcoran JF (1993) Cytotoxicity and dentin permeability of carbamide peroxide and hydrogen peroxide vital bleaching materials, *in vitro Journal of Dental Research* **72(5)** 931-938.

35. Trindade FZ, Ribeiro AP, Sacono NT, Oliveira CF, Lessa FC, Hebling J, & Costa CA (2009) Trans-enamel and trans-dentinal cytotoxic effects of a 35% H_2O_2 bleaching gel on cultured odontoblast cell lines after consecutive applications *International Endodontic Journal* **42(6)** 516-524.

36. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, Chen S, Corpe C, Dutta A, Dutta SK, & Levine M (2003) Vitamin C as an antioxidant: Evaluation of its role in disease prevention *Journal of the American College of Nutrition* **22(1)** 18-35.

37. Rodrigo R, Prat H, Passalacqua W, Araya J, & Bachler JP (2008) Decrease in oxidative stress through supplementation of vitamins C and E is associated with a reduction in blood pressure in patients with essential hypertension *Clinical Science (London)* **114(10)** 625-634.

38. Monti DA, Mitchell E, Bazzan AJ, Littman S, Zabrecky G, Yeo CJ, Pillai MV, Newberg AB, Deshmukh S, & Levine M (2012) Phase I evaluation of intravenous ascorbic acid in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer *PLoS One* **7(1)** e29794.

39. Levine M, Padayatty SJ, & Espey MG (2011) Vitamin C: A concentration-function approach yields pharmacology and therapeutic discoveries *Advances in Nutrition (Bethesda, Md)* **2(2)** 78-88.

40. Graumlich JF, Ludden TM, Conry-Cantilena C, Cantilena LR Jr, Wang Y, & Levine M (1997) Pharmacokinetic model of ascorbic acid in healthy male volunteers during depletion and repletion *Pharmaceutical Research* **14(9)** 1133-1139.

41. Ivanoff CS, Hottel TL, & Garcia-Godoy F (2012) Dielectrophoresis: A model to transport drugs directly into teeth *Electrophoresis* **33(8)** 1311-1321.

42. Meireles SS, Heckmann SS, Leida FL, dos Santos Ida S, Della Bona A, & Demarco FF (2008) Efficacy and safety of 10% and 16% carbamide peroxide tooth-whitening gels: A randomized clinical trial *Operative Dentistry* **33(6)** 606-612.

43. Matis BA, Cochran MA, Wang G, & Eckert GJ (2009) A clinical evaluation of two in-office bleaching regimens with and without tray bleaching *Operative Dentistry* **34(2)** 142-149.

44. Ontiveros JC, & Paravina RD (2009) Color change of vital teeth exposed to bleaching performed with and without supplementary light *Journal of Dentistry* **37(11)** 840-847.

45. Tay LY, Kose C, Herrera DR, Reis A, & Loguercio AD (2012) Long-term efficacy of in-office and at-home bleaching: A 2-year double-blind randomized clinical trial *American Journal of Dentistry* **25(4)** 199-204.

5 DISCUSSÃO

É inerente ao ser humano inquietar-se com aquilo que o desagrada ou faz sofrer, como também é próprio da sua condição questionar ou procurar atingir o que lhe agrada ou satisfaz. Isso leva a busca incessante pelo belo.

Hoje os corpos são esculpidos em academias e centros de estética por meio de cirurgias plásticas, de preenchimentos e aplicações de Botox®. Os cabelos são modificados em sua cor e forma, os rostos são maquiados e as bocas exibem lábios contornados cujos os dentes são perfeitamente corrigidos, clareados ou refeitos com técnicas avançadas de micro-laminados, sendo isso fruto de pesquisas realizadas para satisfazer as necessidades humanas.

A esperança de ver uma face bonita em conjunto com um sorriso harmonioso, impactado pela apresentação de dentes perfeitos, leva profissionais e cientistas a incansáveis pesquisas a fim de satisfazer essas exigências.

Mas, assim como um corpo escultural não se mantém sem exercícios físicos e nem sem sobrecarga muscular que causam dores, assim como nenhuma cirurgia é indolor, também o tratamento odontológico, seja para corrigir ou para atingir o ideal de beleza dos dentes, mais especificamente no caso do clareamento dental, pouco ainda se consegue sem provocar a sensibilidade e desconforto no paciente.

Embora exista a possibilidade de realizarmos um tratamento clareador com produtos oxidantes de menor concentração e que apresenta menor prevalência de dor (Tam[33] 1999), essa técnica é limitada, pois alguns pacientes não se adaptam ao uso da moldeira necessária no procedimento caseiro, e muitas vezes, não podem esperar o tempo de três semanas para perceberem os resultados dos seus dentes clareados (Tay, Kose, Loguercio et al.[11] 2009), limitando assim o uso desse procedimento.

Dessa forma, a outra opção é realizar o clareamento em consultório, utilizando produtos oxidantes de maior concentração, com os quais a variação de cor é observada rapidamente (Tay, Kose, Loguercio et al.[11] 2009; Bernardon, Sartori, Ballarin, et al.[12] 2010; Strobl, Gutknecht, Franzen, et al.[13] 2010; Kossatz, Martins, Loguercio, et al. [14] 2011; Reis, Dalanhol, Cunha, et al. [15] 2011), porém apesar da sua efetividade, 55 a 90% dos pacientes que se submetem a esse procedimento em

consultório, apresentam sensibilidade dental (Haywood[4] 2005, Tay, Kose, Loguercio et al.[11] 2009; Reis, Dalanhol, Cunha, et al. [15] 2011; Kihn[16] 2007; Marson, Sensi, Vieira et al.[17] 2008).

É importante relatar que o efeito de rápida variação de cor e a alta prevalência de sensibilidade também foi observado em nossa pesquisa. Gostaríamos que o resultado que o paciente deseja - dentes brancos e sorriso bonito –, fosse atingido sem que ele precisasse passar pelo processo da dor, ou que fosse menos doloroso, pois para chegar ao ideal de belo, o profissional dispõe de recursos modernos, mas nada há que justifique o desconforto e a dor.

Diferentes alternativas têm sido propostas para minimizar essa sensibilidade (Haywood[4] 2005), como a redução da concentração, da frequência e do tempo de uso do gel clareador de peróxido de hidrogênio (Haywood, Leornard, Nelson et al.[18] 1994; Leonard[19] 1998; Tam[20] 2001, Cardoso, Reis, Loguercio et al.[17] 2006), a utilização de nitrato de potássio (Tay, Kose, Loguercio et al.[11] 2009; Reis et al.[14] 2011), de fluoretos (Armenio, Fitarelli, Armenio et al.[22] 2008) e a administração de um medicamento anti-inflamatório, ibuprofeno 600 mg (Charakorn, Cabanilla, Wagner et al.[23] 2009) utilizado previamente ao tratamento clareador.

Foi assim que procuramos utilizar nos pacientes dos grupos pesquisados medicamentos que primeiramente diminuíssem a prevalência de sensibilidade dental, bem como, diminuíssem também a sua intensidade. No entanto, ao contrário da nossa expectativa, a utilização dos medicamentos anti-inflamatórios ibuprofeno 400 mg e etoricoxibe 60 mg não foram capazes de prevenir, e, nem mesmo, minimizar a sensibilidade dental quando administrados de acordo com a posologia e dose empregadas neste estudo.

Dessa forma, compreendemos que a ineficácia dos medicamentos anti-inflamatórios ibuprofeno 400 mg e etoricoxibe 60 mg na prevenção da sensibilidade dental pode ser atribuída em função de vários fatores:

- Da presença de diferentes mediadores, além da COX - 1 e COX - 2, na inflamação do tecido pulpar causada pelo peróxido de hidrogênio (Holt, Hutchins, Pileggi[34] 2005), como a bradicinina (Lepinski, Hargreaves, Goodies et al.[35] 2000) e a substância P (Rodd, Boissonnade[36] 2000). É válido ressaltar que os medicamentos utilizados nesta pesquisa são anti-inflamatórios que agem especificamente no bloqueio da via COX e não seriam capazes de impedir a produção de outros

mediadores da inflamação.

- Porque a COX - 2 pode não ser expressada na polpa dental imediatamente após ao procedimento de clareamento dental.

- Também pela administração de uma dose baixa dos medicamentos. Isso porque, no mercado, existem doses de ibuprofeno até 600 mg, etoricoxibe com até 120 mg. Nesse sentido, o emprego de doses elevadas poderia ser mais eficiente para a redução da sensibilidade dental.

- Além disso, se a causa da sensibilidade dental for devido ao dano celular, por meio da formação de radicais livres e espécies reativas de oxigênio (Anderson, Chiego, Glickman et al.[37] 1999), os medicamentos anti-inflamatórios, como os utilizados neste estudo, não são capazes de reduzir a experiência de dor por ação antioxidante.

Ademais, ao contrário da nossa prévia expectativa, nem a utilização de um antioxidante ácido ascórbico 500 mg (Vitamina C) foi capaz de evitar a sensibilidade dental, assim como não foi reduzida a sua intensidade pela redução de formação de radicais livres. É importante destacar que isso pode ter ocorrido porque quando o ácido ascórbico é administrado via oral, vários fatores, tais como, a presença do sistema imunológico, a drenagem linfática, a excreção urinária e as características morfológicas da dentina podem modular a sua quantidade no plasma e no fluído extracelular em torno das células pulpares (Padayatty, Sun, Wang et al.[38] 2004), mesmo administrado em doses elevadas, como a que foi utilizada neste estudo, isso não proporcionaria benefícios adicionais (Levine, Padayatti, Espey[39] 2011).

Outra questão que merece ser relatada é que o medicamento ibuprofeno 400 mg, administrado uma hora antes do tratamento clareador, foi capaz de reduzir a intensidade da sensibilidade durante até a primeira hora após o tratamento clareador. O ibuprofeno 400 mg é um anti-inflamatório capaz de bloquear a enzima ciclo-oxigenase (COX) (Suleyman, Demican, Karagoz[25] 2007), responsável pela produção das prostaglandinas (Nakanishi, Shimizu, Hosokawas et al.[40] 2001) que causam dor (Lepinski, Hargreaves, Goodiis et al.[35] 2000; Nakanishi, Shimizu, Hosokawa et al.[40] 2001, Hargreaves[24] 2002). Esse resultado sugere que a inflamação pulpar que ocorre pelo procedimento de clareamento dental causa a liberação de prostaglandinas na polpa dental via COX -1 na primeira hora após o

tratamento clareador.

Em relação aos resultados de alteração da cor, este estudo indicou que todos os grupos apresentaram valores equivalentes, assim como, indicou a melhora significativa na cor dental, com alteração entre 5 e 8 unidades de cor na escala Vita após duas sessões de clareamento, o que comprova a eficácia e a rapidez desse tratamento.

Os resultados desta pesquisa infelizmente não atingiram as nossas expectativas, que era detectar uma redução na prevalência de dor do paciente para 41% entre o Grupo experimental e controle, com a utilização dos medicamentos ibuprofeno 400 mg e etoricoxibe 60 mg; e uma redução na prevalência de dor do paciente para 40% entre o Grupo experimental e controle, com a utilização do ácido ascórbico. Porém, a ineficácia dos medicamentos levou-nos a procurar justificativas que corroboram com ideias para a realização de novos desenhos experimentais, como tamanhos de amostras maiores, a utilização de doses maiores de anti-inflamatório, e ainda, a utilização de uma droga anti-inflamatória de amplo espectro capaz de inibir maior quantidade de mediadores inflamatórios, tais como os glicocorticoides (Greaves[41] 1976).

O mecanismo de ação do peróxido de hidrogênio ainda não é claro, bem como, não são claros os motivos pelos quais esse produto causa sensibilidade pulpar. Assim sendo, ressaltamos que toda pesquisa neste campo deve ser incentivada, mesmo que a resposta não satisfaça as expectativas iniciais, pois pesquisar é um processo de construção do conhecimento que tem como meta principal gerar novos conhecimentos. Conforme a interpretação de Demo[42] (1987), a pesquisa é a atividade científica pela qual descobrimos a realidade e se justifica pelo aprendizado.

6 CONCLUSÃO

Sendo assim, concluímos que os medicamentos ibuprofeno 400 mg, etoricoxibe 60 mg e ácido ascórbico 500 mg (vitamina C) não foram capazes de prevenir e nem mesmo de minimizar a intensidade da sensibilidade dental quando administrados de acordo com a posologia e dose empregadas neste estudo. Apenas o ibuprofeno 400 mg reduziu a intensidade da sensibilidade ocorrida imediatamente após o tratamento clareador. Os medicamentos não afetaram a magnitude alcançada com a técnica de clareamento dental em consultório.

REFERÊNCIAS

1. Joiner A. The tooth colour: a review of the literature. J Dent. 2004 Oct;32(1):3-12.

2. Joiner A. The bleaching of teeth: A review of the literature. J Dent. 2006 Aug;34(7):412-419.

3. Giniger M, MacDonald J, Felix H. The clinical performance of profissionaly dispensed bleaching with added amorphous calcium phosphate. JADA 2005 Mar;136(3):383-392.

4. Haywood VB. Treating sensitivity during tooth whitening. Compend Contin Educ Dent. 2005 Sep;26(9 Suppl 3):11-20.

5. Markowitz K. Pretty painful: Why does tooth bleaching hurt? Med Hypotheses. 2009 May; 74(5):835-840.

6. Cooper JS, Bokmeyer TJ, Bowles WH. Penetration of the pulp chamber by carbamide peroxide bleaching agents. J Endod. 1992 Jul;18(7):315-317.

7. Cohen SC. Human pulpal response to bleaching procedures on vital teeth. J Endod. 1979 May;5(5):134-138.

8. Robertson WD, Melfi RC. Pulpal response to vital bleaching procedures. J Endod. 1980 Jul;6(7):645-649.

9. Schulte JR, Morrissette DB, Gasior EJ, et al. The effects of bleaching application time on the dental pulp. JADA. 1994 Otc;125(10):1330-1335.

10. Leonard RH, Smith LR, Garland GE, et al. Desensitizing agent efficacy during whitening in an at-risk population. J Esthet Restor Dent. 2004 Jan;16(1):49-56.

11. Tay LY, Kose C, Loguercio AD, et al. Assessing the effect of a desensitizing agent used before in-office tooth bleaching. JADA. 2009 Oct;140(10):1245-1251.

12. Bernardon JK, Sartori N, Ballarin A, et al. Clinical performance of vital bleaching techniques. Oper Dent. 2010 Jan-Fev;35(1):3-10.

13. Strobl A, Gutknecht N, Franzen R, Hilgers RD, et al. Laser-assisted in-office bleaching using a neodymium: yttrium-aluminum-garnet laser: an in vivo study. Lasers Med Sci. 2010 Jul;25(4):503-509.

14. Kossatz S, Martins G, Loguercio AD, et al. Tooth sensitivity and bleaching effectiveness of a calcium-containing in-office bleaching gel. Oper Dent. 2012 Dec;143(12):e81-87.

15. Reis A, Dalanhol AP, Cunha TS, et al. Assessment of tooth sensitivity using a desensitizer before light-activated bleaching. Oper Dent. 2011 Jan-Feb;36(1):12-17.

16. Kihn PW. Vital tooth whitening. Dent Clin North Am. 2007 Apr;51(2):319-331.

17. Marson FC, Sensi LG, Vieira LC, et al. Clinical evaluation of in-office dental bleaching treatments with and without the use of light-activation sources. Oper Dent. 2008 Jan-Feb;33(1):15-22.

18. Haywood VB, Leonard RH, Nelson CF, et al. Effectiveness, side effects and long-term status of nightguard vital bleaching. JADA. 1994 Sep;125(9):1219-1226.

19. Leonard RH Jr. Efficacy, longevity, side effects, and patient perceptions of nightguard vital bleaching. Compend Contin Educ Dent. 1998 Aug;19(8):766-774.

20. Tam L. Effect of potassium nitrate and fluoride on carbamide peroxide bleaching. Quintessence Int. 2001 Nov-Dec;32(10):766-770.

21. Cardoso PC, Reis A, Loguercio A, et al. Clinical Effectiveness and Tooth Sensitivity Associated With Different Bleaching Times for a 10 Percent Carbamide Peroxide Gel. JADA 2006 Oct;41(10):1213-1220.

22. Armenio RV, Fitarelli F, Armenio MF, et al. The effect of fluoride gel use on bleaching sensitivity: a double-blind randomized controlled clinical trial. JADA. 2008 May; 139(5):592-597.

23. Charakorn P, Cabanilla LL, Wagner WC, et al. The effect of preoperative ibuprofen on tooth sensitivity caused by in-office bleaching. Oper Dent. 2009 Mar-Apr;34(2):131-135.

24. Hargreaves KM. Pain mechanisms of the pulp dentin complex In: Seltzer and Bender's Dental Pulp. Quintessence Publishing. 2002:181-256.

25. Suleyman H, Demircan B, Karagoz Y. Anti-inflamatory and side effects of cyclooxygenase inhibitor. Pharmacol Rep. 2007 May-Jun;59(3):247-258.

26. Costa CAS, Riehl H, Kina JF, et al. Human pulp responses to in-office tooth bleaching. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Apr;109(4):e59-e64.

27. Kanno S, Ishikawa M, Takayanagi M, et al. Characterization of hydrogen peroxide-induced apoptosis in mouse primary cultured hepatocytes. Biol Pharm Bull. 2000 Jan; 23(1):37–42.

28. Calabrese V, Lodi R, Tonon C, et al. Oxidative stress, mitochondrial dysfunction and cellular stress response in Friedreich's ataxia. J Neurol Sci. 2005 Jun;233(1-2):145-162.

29. Kanno S, Shouji A, Asou K, et al. Effects of naringin on hydrogen peroxide-induced cytotoxicity and apoptosis in P388 cells. J Pharmacol Sci. 2003 Jun; 92(4): 166-70.

30. Schulz KF, Altman DG, Moher D. CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. Brit Med J. 2010 Mar;8:1–9.

31. Jensen, Karolpy, Braver. The measurement of clinical pain intensity: a comparioson of six methods. Pain. 1986 Oct;27(1):117-126.

32. American Dental Association, Counsil on Scientific Affairs American Dental Association Program Guideline: Dentin and Enamel Adhesive Matirials. 2001 Jun: 1-12.

33. Tam L. Clinical trial of three 10% carbamide peroxide bleaching products. J Can Dent Assoc. 1999 Apr;65(4):201-205.

34. Holt CI, Hutchins MO, Pileggi R. A real time quantitative PCR analysis and correlation of COX-1 and COX-2 enzymes in inflamed dental pulps following administration of three different NSAIDs. J Endod. 2005 Nov;31(11):799-804.

35. Lepinski AM, Hargreaves KM, Goodis HE, et al. Bradykinin levels in dental pulp by microdialysis. J Endod. 2000 Dec;26(12):744-747.

36. Rodd H, Boissonnade F. Substance P expression in human tooth pulp in relation to caries and pain experience. Eur J Oral Sci. 2000 Dec;108(6):467-474.

37. Anderson DG, Chiego DJ, Glickman GN, et al. A Clinical Assessment of the Effects of 10% Carbamide Peroxide Gel on Human Pulp Tissue. J Endod. 1999 Apr;25(4):247-250.

38. Padayatty SJ, Sun H, Wang Y, et al. Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann Intern Med. 2004 Apr;140(7):533-537.

39. Levine M, Padayatty SJ, Espey MG. Vitamin C: a concentration-function approach yields pharmacology and therapeutic discoveries. Adv Nutr. 2011 Mar;2(2):78-88.

40. Nakanishi T, Shimizu H, Hosokawa Y, et al. An immunohistological study on cyclooxygenase-2 in human dental pulp. J Endod. 2001 Jun;27(6):385-388.

41. Greaves MW. Anti-inflammatory action of corticosteroids. Postgrad Med J. 1976 Oct;52(612):631-33.

42. Demo P. Introdução à metodologia da ciência. 2. ed. São Paulo: Atlas, 1987.

ANEXO A

Aprovação do projeto de pesquisa na Comissão de Ética em Pesquisa da Universidade Estadual de Ponta Grossa

UEPG
UNIVERSIDADE ESTADUAL DE PONTA GROSSA

PROPESP
Pró-Reitoria de Pesquisa e Pós-Graduação

COEP
COMISSÃO DE ÉTICA EM PESQUISA

PARECER Nº 21/2011
Protocolo: 17836/10

No dia 31 de março de 2011, a Comissão de Ética em Pesquisa, **APROVOU** o protocolo de pesquisa intitulado **"O Efeito de diferentes medicamentos na prevenção da sensibilidade causada pelo clareamento dental"** de responsabilidade da pesquisadora **Alessandra Reis.**

Conforme Resolução CNS 196/96, solicitamos que sejam apresentados a esta Comissão, relatórios sobre andamento da pesquisa, conforme modelo (http://www.uepg.br/coep/).

Data para entrega do relatório parcial: 31 de março de 2012.
Data para entrega do relatório Final: 31 de março de 2013.

Ponta Grossa, 01 de abril de 2011.

UNIVERSIDADE ESTADUAL DE PONTA GROSSA
COMISSÃO DE ÉTICA EM PESQUISA - COEP

Prof. Dr. Ulisses Coelho
Coordenador

Av. Carlos Cavalcanti, 4748 - CEP 84030-900 - Ponta Grossa - PR - BRASIL
Bloco M Sala 12 - Campus Universitário em Uvaranas
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ANEXO B

Termo de Consentimento Livre e Esclarecido

Universidade Estadual de Ponta Grossa
Pró-Reitoria de Pesquisa e Pós-Graduação

Av.: Gen. Carlos Cavalcanti, 4748 CEP: 84030-900
Campus Uvaranas Ponta Grossa Fone: 42.220.3262 e-mail: *pesquisa@uegp.br*

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

1. Título da Pesquisa: ***O efeito de diferentes medicamentos na prevenção da sensibilidade causada pelo clareamento dental.***

2. Pesquisadores: Profa. Dra. Stella Kossatz Pereira; Profa. Dra. Alessandra Reis; Prof. Dr. Alessandro Loguércio; Aluna Doutoranda Eloisa Andrade de Paula.

3. Proposição: Verificar a capacidade de diferentes medicamentos, na prevenção da sensibilidade dental causada pelo clareamento de consultório com peróxido de hidrogênio 35%.

4. Procedimentos do Experimento: Esta técnica de clareamento é realizada no consultório odontológico onde o cirurgião-dentista aplica o produto clareador sobre os dentes com vitalidade pulpar. Para proteção da gengiva será utilizado uma barreira gengival (Top Dam, FGM) que garante que apenas os dentes selecionados entrarão em contato com o gel clareador. Todo este procedimento leva aproximadamente 1 hora, e serão realizadas 2 sessões com intervalo de 1 semana entre elas. Alguns pacientes durante o clareamento apresentam sensibilidade nos dentes ocasionada pela ação do produto. No caso de ocorrer sensibilidade severa serão feitas aplicações de dessensibilizante medicando o paciente com paracetamol 750 mg.

5. Local da pesquisa: O tratamento e exame clínico serão realizados na Clínica Odontológica do Curso de Odontologia da Universidade Estadual de Ponta Grossa. Durante este período os voluntários serão acompanhados pelos pesquisadores para a verificação de qualquer efeito adverso.

6. Resultados esperados: A resposta da pesquisa pode trazer benefício clínico, pois se espera que a administração prévia dos medicamentos reduza ou previna a sensibilidade pós-operatória decorrente da realização da técnica de clareamento dental de consultório.

7. Análise crítica dos riscos e benefícios*:* A utilização de qualquer agente químico utilizado para o clareamento dental pode ocasionar efeitos adversos como sensibilidade, ardência, descamação e ulceração das mucosas bucais, dependendo da sensibilidade individual. Após o relato de qualquer efeito adverso (exceto sensibilidade), o tratamento com o clareador será imediatamente suspenso, com a retirada do sujeito da pesquisa. Quanto aos benefícios, os pacientes da pesquisa terão seus dentes clareados, e receberão gratuitamente o clareamento, bem como o agente utilizado para o tratamento de uma eventual sensibilidade. Os pacientes podem reclamar de algumas reações adversas dos medicamentos como tontura, inchaço das pernas e/ou pés, fraqueza e cansaço, pressão alta, enjoos, azia, desconforto no estômago e dor de cabeça, diarreia ou prisão de ventre, náuseas ou vômitos, úlcera de estômago ou duodeno que provocam eliminação de sangue vivo, ou fezes como borra de café, zumbido no ouvido ou tontura, inchaço das pernas, reações alérgicas tipo urticária e coceira. Serão dadas todas as informações sobre qualquer

tipo de problema, formas de tratamento, encaminhamento e acompanhamento do adequado tratamento na clínica de pós-graduação da UEPG.

8. ***Forma de acompanhamento e assistência e garantia de esclarecimentos:*** Os indivíduos terão garantia de que receberão esclarecimento de qualquer dúvida acerca dos procedimentos, riscos, benefícios e outros assuntos relacionados com a pesquisa. Os pesquisadores responsáveis assumem o compromisso de proporcionar informação atualizada obtida durante o estudo, ainda que esta possa afetar a vontade do indivíduo em continuar participando dele.

9. **Retirada do consentimento:** Os sujeitos têm a liberdade de se recusar a participar da pesquisa ou de retirar seu consentimento a qualquer momento, sem sofrer qualquer tipo de prejuízo, ou represálias de qualquer natureza.

10. ***Garantia de sigilo:*** Os pesquisadores se comprometem a resguardar todas as informações individuais, tratando-as com impessoalidade e não revelando a identidade do sujeito que as originou.

11. Formas de ressarcimento de despesas e de indenização: Os indivíduos não terão qualquer despesa. Para o tratamento de efeitos adversos os custos estão previstos no orçamento do projeto.

12. Consentimento pós-informação

Eu, _____, certifico que tendo lido as informações acima e suficientemente esclarecido de todos os itens, pelos pesquisadores clínicos responsáveis: Eloisa Andrade de Paula, Stella Kossatz Pereira, Alessandra Reis, Alessandro Loguércio, Daniel Fernandes, estou plenamente de acordo com a realização do experimento. Assim, eu concordo em participar como voluntário do trabalho de pesquisa, exposto acima.

Certifico também ter recebido uma cópia deste Termo de Consentimento Livre e Esclarecido.

Ponta Grossa, _____ de _____ de 2010.
Nome: _____
Assinatura: _____

1ª via da instituição, 2ª via do sujeito da pesquisa

Para entrar em contato com os pesquisadores:
Eloisa Andrade de Paula (42) 9104-0000
Alessandra Reis (42) 3220-3741
Stella Kossatz Pereira (42) 3220-3741
Daniel Fernandes (42) 3220-3120
Alessandro Loguércio (42) 3220-3741

ATENÇÃO: A sua participação em qualquer tipo de pesquisa é voluntária. Em caso de dúvida quanto aos seus direitos, entre em contato com a Comissão de Ética em Pesquisa da UEPG. Endereço – Av. Carlos Cavalcanti, n.4748, Bloco M, Sala 12, CEP- 84030-900 – Ponta Grossa – PR. Fone: (42) 3220-3108. e-mail: coep@uepg.br.

ANEXO C

TERMOS DE AUTORIZAÇÃO PARA PUBLICAÇÃO DE ARTIGOS CIENTÍFICOS PARA A DEFESA DA TESE DE DOUTORADO

ARTIGOS PUBLICADOS NA REVISTA OPERATIVE DENTSTRY

The effect of perioperative ibuprofen use on tooth sensitivity caused by in-office bleaching.

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

Dear Dr. de Paula,

Thank you for your email.

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Best regards,

Kevin Matis

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