UNIVERSIDADE PAULISTA

IMPACTO DA ASSOCIAÇÃO DE CAFEÍNA E NICOTINA NA PROGRESSÃO DA PERIODONTITE EXPERIMENTAL

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Paulista – UNIP, para obtenção do título de Mestre em Odontologia.

ISIS GARCIA DIAS FERREIRA

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DEDICATÓRIA

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IMPACTO DA ASSOCIAÇÃO DE CAFEÍNA E NICOTINA NA PROGRAÇÃO DA PERIODONTITE EXPERIMENTAL

RESUMO

O objetivo deste estudo foi avaliar o efeito da associação de cafeína (CAF) e nicotina (NIC) sobre a progressão da periodontite experimental em ratos. Para tanto, foram utilizados 40 ratos machos Wistar, divididos em 4 grupos: 1- Grupo placebo (PL) (n=10): água destilada; 2- Grupo NIC (n=10): nicotina; 3- Grupo CAF (n=10): cafeína; 4- Grupo CAF+NIC (n=10): cafeína e nicotina. A água destilada e a cafeína (30 mg/kg) foram administradas via gavagem, diariamente, por 45 dias previamente à indução da periodontite experimental e por 15 dias após. A nicotina (1.67 mg/kg/d) foi administrada por meio de injeção intraperitoneal durante o mesmo período dos outros tratamentos. A periodontite experimental foi induzida por meio de colocação de ligaduras em um dos primeiros molares inferiores dos animais. A eutanásia dos animais ocorreu 15 dias após a indução da periodontite experimental. As mandíbulas foram removidas para análise morfométrica da perda óssea alveolar e o peso dos animais foi registrado no baseline (no dia do início dos tratamentos), 15 e 45 dias após o início dos tratamentos. A análise morfométrica mostrou que o grupo PL apresentou menor perda óssea quando comparado aos outros grupos, no lado ligado ($p<0.05$). Maior perda óssea foi observada no grupo CAF+NIC, quando comparada ao grupo placebo e ao grupo NIC, enquanto CAF e NIC apresentaram isolados maior perda óssea comparada ao PL, no lado não ligado ($p<0.05$). O grupo CAF+NIC não teve ganho de peso ao longo do tempo quando comparado aos outros grupos ($P<0.05$). Pode-se concluir que tanto a nicotina, como a cafeína em altas doses e sua associação aumentam a progressão da periodontite experimental. A associação de cafeína e nicotina tem efeito destrutivo sobre o tecido ósseo inclusive na ausência da periodontite experimental, bem como sobre o ganho de peso dos animais.

Palavras chave: Periodontite experimental; Perda óssea alveolar; Cafeína; Nicotina.

IMPACT OF THE ASSOCIATION OF CAFFEINE AND NICOTINE ON THE PROGRESSION OF EXPERIMENTAL PERIODONTITIS

ABSTRACT

The aim of this study was to evaluate the effect of the association of caffeine and nicotine on the progression of experimental periodontitis in rats. For this purpose, 40 male Wistar rats were used, divided into 4 groups: 1- Placebo group (PL) (N=10): distilled water; 2- Nicotine Group (NIC) (N=10): nicotine; 3- Caffeine group (CAF) (N=10): caffeine; 4- Caffeine + nicotine group (CAF+NIC) (N=10): caffeine and nicotine. Distilled water and caffeine (30 mg/kg) were administered via gavage daily, for 45 days prior to the induction of experimental periodontitis and for 15 days afterwards. Nicotine (1.67 mg/kg/d) was administered via intraperitoneal injection during the same period as the other treatments. Experimental periodontitis was induced by placing ligatures on one of the lower first molars of the animals. The euthanasia of the animals occurred 15 days after the induction of experimental periodontitis. The mandibles were removed for morphometric analysis of alveolar bone loss and the weight of the animals was recorded at baseline (on the day of the beginning of the treatments), 15 and 45 days after the beginning of the treatments. Morphometric analysis showed that the PL group had less bone loss when compared to the other groups, on the ligated side ($p<0.05$). Higher bone loss was observed in CAF+NIC group, when compared to the placebo group and the NIC group, on the non-ligated side ($p<0.05$), while CAF/NIC isolated had higher bone loss compared to PL (0.05). CAF+NIC group did not gain weight over time when compared to the other groups ($P<0.05$). It can be concluded that both nicotine and caffeine in high doses and their association increase the progression of experimental periodontitis. The association of caffeine and nicotine has a destructive effect on bone tissue even in the absence of experimental periodontitis, as well as on weight gain in animals.

Key-words: Experimental periodontitis; Alveolar bone loss; Caffeine; Nicotine.

SUMÁRIO

1 INTRODUÇÃO

A doença periodontal é uma infecção que conduz à destruição do tecido de suporte, induzindo a produção local de mediadores imunoinflamatórios em resposta a periodontopatógenos e seus produtos (Kinane et al. 2011). Desta forma, embora o agente etiológico da periodontite seja o biofilme bacteriano específico, existe um consenso de que a resposta do hospedeiro frente ao desafio bacteriano é primordial para o desenvolvimento da doença. Estudos tem demonstrado que fatores de risco sistêmico (diabetes e osteoporose) e ambientais, como tabagismo, podem modular progressão e severidade da doença (Albandar, 2002; Johnson & Hill, 2004, Ryan et al., 2003; Lerner 2006).

A nutrição tem consequências importantes para a saúde e pode contribuir para o desenvolvimento e progressão de doenças crônicas, incluindo doenças cardiovasculares, diabetes e câncer (AAP 2005). Nesse contexto, a cafeína, 1,3,7-trimetilxantina, é um dos compostos psicoativos mais frequentemente ingeridos no mundo, estando presente em bebidas, alimentos e medicamentos (Heckman et al., 2010), sendo que 80% da população mundial consome um produto contendo cafeína, pelo menos 1 vez ao dia. Adicionalmente, estudos prévios evidenciaram o efeito negativo de altas doses de cafeína sobre a progressão da periodontite experimental (Bezerra et al., 2008), sobre o reparo ósseo inicial de defeitos críticos (Duarte et al., 2009) e alvéolos pós- exodontia (Macedo et al. 2015), e no metabolismo ósseo (viabilidade das células ósseas, histomorfometria, e índice mineral ósseo) (Cooper et al, 1992; Chen & Whitford, 1999; Rapuri et al, 2001; Huang et al, 2002; Tsuang et al., 2006; Rapuri et al., 2007).

O tabagismo é considerado um dos fatores de risco para as doenças periodontais, influenciando negativamente o estabelecimento e progressão dessas (Meulman et al. 2013, Johannsen et al. 2014) e desempenhando um importante papel na exacerbação da resposta imunoinflamatória do hospedeiro frente ao desafio bacteriano (Johannsen et al. 2014). Com relação à doença periodontal frente à presença do fumo, há evidências de que a exposição de leucócitos e outras células à fumaça de cigarro resulte em diversos efeitos relacionados à patogênese da doença periodontal em fumantes, ativando células inflamatórias e aumentando os níveis de marcadores inflamatórios (Loos et al. 2004, Barbieri et al. 2011). O tabagismo causa também uma elevação nos níveis circulantes e locais de enzimas responsáveis pela degeneração tecidual, como a matriz de metaloproteinases (Liu et al. 2006, Ozcaka et al. 2011). Adicionalmente, fumantes têm aumentada taxa de RANKL⁄OPG na saliva e no soro, principalmente devido aos reduzidos níveis de OPG (Lappin et al. 2007, Ozcaka et al. 2010).

Outro mecanismo associado ao papel do tabagismo na destruição periodontal pode ser relacionado ao aumento do estresse oxidativo em pacientes fumantes (Matthews et al. 2011).

O consumo de cafeína é fortemente associado ao tabagismo tanto em estudos epidemiológicos (Brice & Smith, 2006; Istvan & Matarazzo, 1984, Swanson et al, 2009; Gurpegui et al., 2004), como em estudos clínicos populacionais (Reich et al., 2008; Emurian et al., 1982). Uma análise mendeliana de 2017, mostra que o tabagismo pesado aumenta causalmente o consumo de café. Isto é consistente com um metabolismo mais rápido da cafeína pelos tabagistas, mas poderia também refletir um efeito comportamental do fumo no consumo de café (Bjørngaard et al., 2017). Os tabagistas não só tendem a beber cafeína e os consumidores de cafeína tendem a fumar, estes comportamentos ocorrem frequentemente ao mesmo tempo (Emurian et al., 1982; Lane et al., 1996; Shiffman et al., 2002). Shiffman et al. (2002) observaram que as probabilidades de fumar aumentaram 55%, em média, durante os períodos de consumo de cafeína. Uma justificativa é que fumar aumenta o metabolismo da cafeína, exigindo que os tabagistas consumam mais cafeína para alcançar os efeitos desejados (Brown et al., 1988, Swanson et al., 1997). Além disso, os tabagistas também relatam que a palatabilidade dos cigarros é aumentada pelo consumo de cafeína, sugerindo que alguns aspectos da motivação para fumar são influenciados pelo consumo de cafeína (McClernon et al., 2007).

Diversos mecanismos comportamentais e farmacológicos foram sugeridos para explicar a associação entre o consumo de cafeína e o tabagismo. Uma explicação aceitável é que a cafeína e o tabaco podem ser utilizados de forma intermutável, devido aos seus efeitos estimulantes semelhantes. Embora por diferentes vias, tanto a cafeína como a nicotina aumentam a atividade dopaminérgica (Tanda & Goldberg, 2000) e a sua popularidade deve-se em parte à sua propensão para aumentar a energia, concentração, alerta e disposição. Os indivíduos que apreciam e procuram estes efeitos podem utilizar qualquer uma das substâncias para estes resultados. Os protocolos experimentais duplo-cego em humanos também demonstraram que a cafeína e a nicotina produzem efeitos psicoativos dose-dependente, positivos e subjetivos semelhantes quando administrados individualmente (Garrett & Griffiths, 2001; Jones & Griffiths, 2003). Além disso, animais e humanos treinados para discriminar a nicotina do placebo são mais susceptíveis a identificar um placebo como contendo nicotina se este seguir da administração aguda de cafeína, o que sugere que os sinais interoceptivos de nicotina e cafeína são semelhantes (Gasior et al., 2002; Duka et al., 1998).

Desta forma, considerando-se o efeito negativo exercido tanto pelo alto consumo de cafeína, como pelo tabagismo sobre os tecidos periodontais e ausência de estudos que

investiguem o efeito combinado dessas substâncias, o presente projeto tem como objetivo avaliar o papel da combinação de ambas as substâncias na modulação da periodontite experimental em animais, por meio de análise morfométrica da perda óssea alveolar.

2 ARTIGO

IMPACT OF THE ASSOCIATION OF CAFFEINE AND NICOTINE ON THE PROGRESSION OF EXPERIMENTAL PERIODONTITIS

ABSTRACT

The aim of this study was to evaluate the effect of the association of caffeine and nicotine on the progression of experimental periodontitis in rats. For this purpose, 40 male Wistar rats were used, divided into 4 groups: 1- Placebo group (PL) (N=10): distilled water; 2-Nicotine Group (NIC) (N=10): nicotine; 3- Caffeine group (CAF) (N=10): caffeine; 4- Caffeine + nicotine group (CAF+NIC) (N=10): caffeine and nicotine. Distilled water and caffeine (30 mg/kg) were administered via gavage daily, for 45 days prior to the induction of experimental periodontitis and for 15 days afterwards. Nicotine (1.67 mg/kg/d) was administered via intraperitoneal injection during the same period as the other treatments. Experimental periodontitis was induced by placing ligatures on one of the lower first molars of the animals. The euthanasia of the animals occurred 15 days after the induction of experimental periodontitis. The mandibles were removed for morphometric analysis of alveolar bone loss and the weight of the animals was recorded at baseline (on the day of the beginning of the treatments), 15 and 45 days after the beginning of the treatments. Morphometric analysis showed that the PL group had less bone loss when compared to the other groups, on the ligated side ($p<0.05$). Higher bone loss was observed in the CAF+NIC group, when compared to the placebo group and the NIC group, while CAF/NIC isolated had higher bone loss compared to PL on the non-ligated side ($p<0.05$). The CAF+NIC group did not gain weight over time when compared to the other groups ($P<0.05$). It can be concluded that both nicotine and caffeine in high doses and their association increase the progression of experimental periodontitis. The association of caffeine and nicotine has a destructive effect on bone tissue even in the absence of experimental periodontitis, as well as on weight gain in animals.

Key-words: experimental periodontitis, alveolar bone loss, caffeine, nicotine.

INTRODUCTION

Periodontitis is an infection that leads to periodontal tissue destruction, inducing the local production of immunoinflammatory mediators in response to periodontopathogens and their products (Kinane et al. 2011). Thus, although the etiological agent of periodontitis is the specific bacterial biofilm, there is a consensus that the host response to the bacterial challenge is essential for the development of the disease. Studies have shown that systemic and environmental risk factors, such as smoking, diabetes and osteoporosis, can modulate disease progression and severity (Albandar, 2002; Johnson & Hill, 2004, Ryan et al., 2003; Lerner 2006).

Nutrition has important health consequences and may contribute to the development and progression of chronic diseases, including cardiovascular disease, diabetes, and cancer (AAP 2005). In this context, caffeine, 1,3,7-trimethylxanthine, is one of the most frequently ingested psychoactive compounds in the world, being present in beverages, foods and medicines (Heckman et al., 2010), with 80% of the world's population consuming a caffeine-containing product at least once a day. Additionally, previous studies have shown the negative effect of high doses of caffeine on the progression of experimental periodontitis (Bezerra et al., 2008), on the initial bone repair of critical defects (Duarte et al., 2009) and post-extraction sockets (Macedo et al. 2015), and on bone metabolism (bone cell viability, histomorphometry, and bone mineral index) (Cooper et al, 1992; Chen & Whitford, 1999; Rapuri et al, 2001; Huang et al, 2002; Tsuang et al., 2006; Rapuri et al., 2007).

Smoking is considered one of the risk factors for periodontal diseases, negatively influencing their establishment and progression (Meulman et al. 2013, Johannsen et al. 2014) and playing an important role in exacerbating the host's immune-inflammatory response to bacterial challenge (Johannsen et al. 2014). With regard to periodontal disease in the presence of tobacco, there is evidence that the exposure of leukocytes and other cells to cigarette smoke results in several effects related to the pathogenesis of periodontal disease in smokers, activating inflammatory cells and increasing the levels of inflammatory markers (Loos et al 2004, Barbieri et al 2011). Smoking also causes an increase in circulating and local levels of enzymes responsible for tissue degeneration, such as matrix metalloproteinases (Liu et al. 2006, Ozcaka et al. 2011). Additionally, smokers have increased RANKL⁄OPG in saliva and serum, mainly due to reduced OPG levels (Lappin et al. 2007, Ozcaka et al. 2010). Another mechanism associated with the role of smoking in periodontal destruction may be related to increased oxidative stress in smokers (Matthews et al. 2011).

Caffeine consumption is strongly associated with smoking both in epidemiological studies (Brice & Smith, 2006; Istvan & Matarazzo, 1984, Swanson et al, 2009; Gurpegui et al., 2004) and in population clinical studies (Reich et al., 2008; Emurian et al., 1982). A Mendelian analysis shows that heavy smoking causally increases coffee consumption. This is consistent with a faster metabolism of caffeine by smokers, but could also reflect a behavioral effect of smoking on coffee consumption (Bjørngaard et al., 2017). Not only do smokers tend to drink caffeine and caffeine consumers tend to smoke, but these behaviors also often occur at the same time (Emurian et al., 1982; Lane et al., 1996; Shiffman et al., 2002). Shiffman et al. (2002) found that the chances of smoking increased by an average of 55% during periods of caffeine consumption. One explanation is that smoking increases caffeine metabolism, requiring smokers to consume more caffeine to achieve the desired effects (Brown et al., 1988, Swanson et al., 1997). In addition, smokers also report that cigarette palatability is increased by caffeine consumption, suggesting that some aspects of smoking motivation are influenced by caffeine consumption (McClernon et al., 2007).

Several behavioral and pharmacological mechanisms have been suggested to explain the association between caffeine consumption and smoking. One acceptable explanation is that caffeine and tobacco can be used interchangeably due to their similar stimulant effects. Although in different ways, both caffeine and nicotine increase dopaminergic activity (Tanda & Goldberg, 2000) and their popularity is due in part to their propensity to increase energy, concentration, alertness, and disposition. Individuals who enjoy and seek these effects can use either substance for these results. Double-blind experimental protocols in humans have also demonstrated that caffeine and nicotine produce psychoactive effects of whether-dependent, positive, and similar subjective when administered individually (Garrett & Griffiths, 2001; Jones & Griffiths, 2003). Furthermore, animals and humans trained to discriminate nicotine from placebo are more likely to identify a placebo as containing nicotine if it follows acute caffeine administration, which suggests that the interoceptive signals of nicotine and caffeine are similar (Gasior et al., 2002; Duka et al., 1998).

Thus, considering the negative effect exerted by both high caffeine consumption and smoking on periodontal tissues and the absence of studies investigating the combined effect of these substances, the present project aims to evaluate the role of the combination of both substances in the modulation of experimental periodontitis in animals, through morphometric analysis of alveolar bone loss.

MATERIAL AND METHODS

Animals

Forty adults male Wistar rats (200–300 g- Butantan Institute, Butantan, Sao Paulo, Brazil) were used. The rats were acclimatized for 15 days before use and they were kept in temperature-controlled cages (5 animals/cage), exposed to a 24-h light–dark cycle of equal time, and had free access to water and food ad libitum (Labina, Purina, Paulínia, Sao Paulo, Brazil) in the Bioterium of Paulista University. The experimental procedure was approved by the Paulista University Institutional Animal Care and Use Committee (6849150222 CEP/ICS/UNIP).

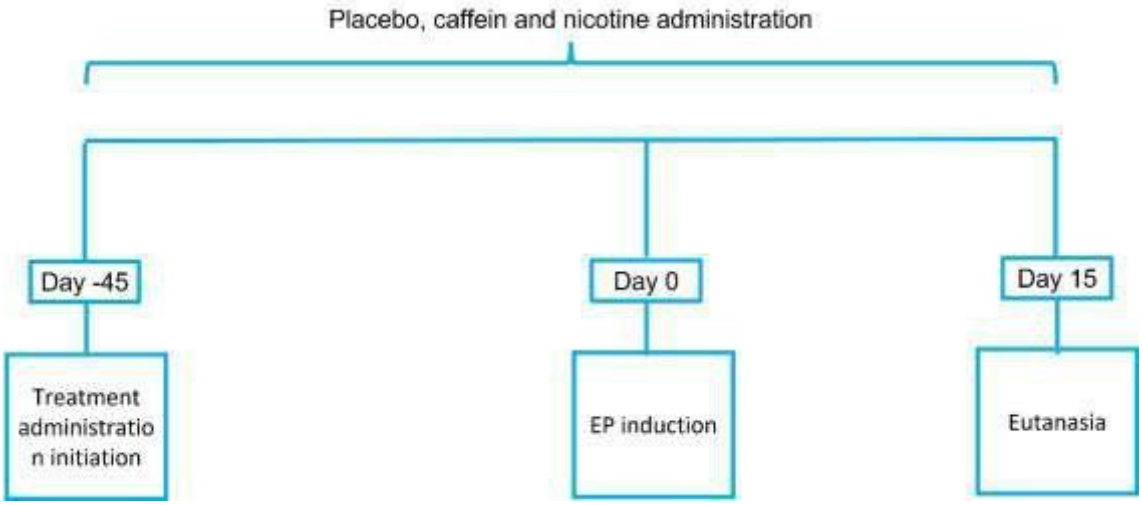
Experimental design

Treatment groups

The experimental design can be observed in Figure 1. The animals were assigned to the following groups: 1- Placebo Group (PL) (N=10): the animals received distilled water; 2- Nicotine Group (NIC) (N=10): the animals received nicotine application; 3- Caffeine Group (CAF) (N=10): the animals received caffeine; 4- Caffeine + nicotine Group (CAF + NIC) (N=10): the animals will receive caffeine and nicotine. The number of animals included in the present study was based on previous studies that had found significant differences in the levels of bone loss (Molez et al., 2020, Corrêa et al., 2018, Ribeiro et al., 2017).

Caffeine (30 mg/kg) (Dal-Fabbro et al., 2021) and placebo were daily administrated by gavage for 60 days (45 days before experimental periodontitis induction and 15 days after). Nicotine solution (1,67 mg/kg - Lee SH et al., 2021) (N3876, Sigma-Aldrich, USA) was daily administered intraperitonially. The solutions of CAF (C0750– Sigma-Aldrich Ltda, Sao Paulo, SP, Brazil) and NIC were prepared using distilled water. The placebo solution consisted also of distilled water. Administration of substances were done always in the morning. The caffeine dose simulates excessive caffeine consumption (Bezerra et al. 2008, Duarte et al. 2009, Bezerra et al. 2013, Bastos et al. 2014). Nicotine dose is representative of doses consumed by humans who smoke 20 cigarettes/day, containing 2.0 mg of nicotine each (Nociti, et al., 2001).

Figure 1. Schematic illustration of the experimental design.



Experimental periodontitis model

To induce EP, the first mandibular molars in each animal were assigned to receive a cotton ligature (Coats Corrente no. 10, Sao Paulo, SP, Brazil) knotted subgingivally at the cementoenamel junction (CEJ). The ligatures were kept in position in order to allow biofilm accumulation over 15 days thereby providing for initiation of EP (Pimentel et al., 2020). This procedure was performed under general anesthesia by the intramuscular administration of ketamine hydrochloride (0.5 mL/kg) and xylazine hydrochloride (10 mg/kg). Fifteen days after EP induction the animals were euthanized by deepening of anesthesia. The mandibula were excised for morphometric analysis of alveolar bone loss.

Measurement of alveolar bone loss by morphometry

Linear measurement of cementum enamel junction – alveolar bone (CEJ-AB) distance

As previously described, after gingival dissection, the mandibula were de-fleshed by immersing the specimens in 8% sodium hypochlorite for 4h. The samples were washed in running water and dried with compressed air. To discriminate the CEJ, 1% aqueous methylene blue solution was applied for 1 min followed by a wash in running water (Molez et al., 2020, Pimentel et al., 2020, Corrêa et al., 2018, Ribeiro et al 2017, Corrêa et al., 2017). Photographs were taken with a 6.1-megapixel digital camera (EOS 40D; Canon, New York, NY, USA) placed on a tripod to keep the camera parallel to the ground at the minimal focal distance thereby insuring reproducibility of image acquisition. The specimens were stabilized in wax with their

occlusal planes parallel to the ground and long axes perpendicular to the camera. Photographs of the palatal aspects were taken, and representative linear alveolar bone loss was assessed on the buccal surface of the molars by measuring the distance of the CEJ from the alveolar bone crest at three equally distant sites using an image analysis system (Image-Pro; Media Cybernetics, Silver Spring, MD, USA). The average CEJ–ABC distance of each tooth was calculated. To validate measurement conversions, all specimens were photographed alongside a millimeter ruler (Corrêa et al., 2018, Corrêa et al., 2017, Casati et al., 2013). The measurements were performed after intra-examiner calibration by evaluating 10 images not taken for this study. The single examiner (IG) was blinded to the experimental group identities when performing morphometric measurements. The examiner took the linear measurements of all photographs twice within 24 hours. The intra-class correlation was 98%.

Statistical Analysis

Statistical analysis was performed using statistical program (SAS software Program Release 9.3; Cary, NC, USA). The data were examined for normality using the Shapiro-Wilk test and homogeneity of variance by Levene's test. The data that achieved normality were analyzed using parametric methods. The data were then analyzed ANOVA/ Tukey tests for comparison between the experimental groups. A significance level of 5% was adopted for all evaluations. The primary outcome was defined as linear alveolar bone loss.

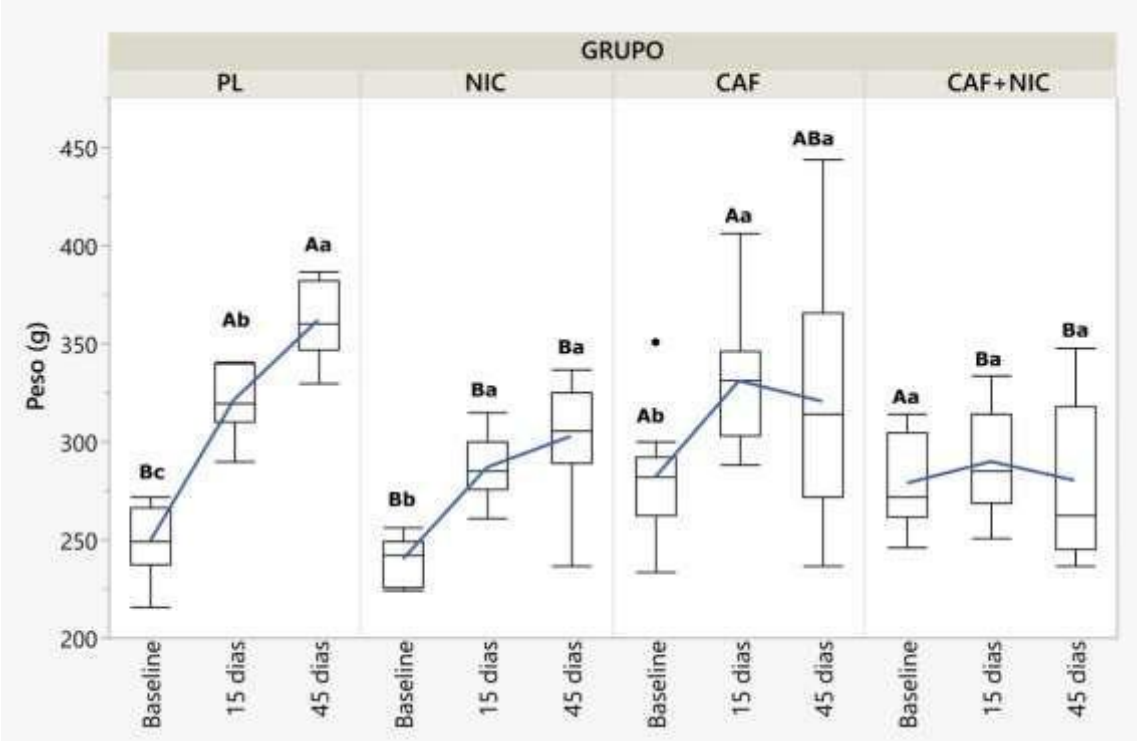
RESULTS

Clinical analysis

As described above, the number of animals included in the present study was based on previous studies that had found significant differences in the levels of alveolar bone loss. The animals that received the association of CAF and NIC did not have weight gain throughout the time, compared to the other groups ($p<0.05$). PL group presented weigh gain over the time ($p<0.05$), while NIC and CAF gained weigh at 15 days of drug ingestion ($p<0.05$) and maintained the weigh until the end of the experiment ($p>0.05$). Animals presented weigh difference at baseline (at the day of treatment initiation) with CAF and NIC+CAF group presenting higher weight compared to PL and NIC ($P<0.05$). Even though NIC+CAF and NIC had lower weigh than PL at 15 days of drug ingestion compared to ($p<0.05$) (Figure 2). One animal from NIC+CAF group was lost. However, three other animals from the same group had

their mandibular bone fractured due to bone fragility and the specimens could not be used for morphometric analysis. During the euthanasia, clinical examination revealed signs of gingival inflammation, including color/volume changes and bleeding around the ligated teeth of all groups. The study followed the ARRIVE guidelines (Sergides et al., 2016).

Figure 2. Weight of Animals in the Different Groups and Experimental Periods.



Data are means (SD). Different letters indicate statistical significance. Uppercase letter compares difference between groups (Anova two-way/ Tukey Test; $p<0.05$); Lowercase letters compare difference within same group (Anova two-way/ Tukey Test; $p<0.05$).

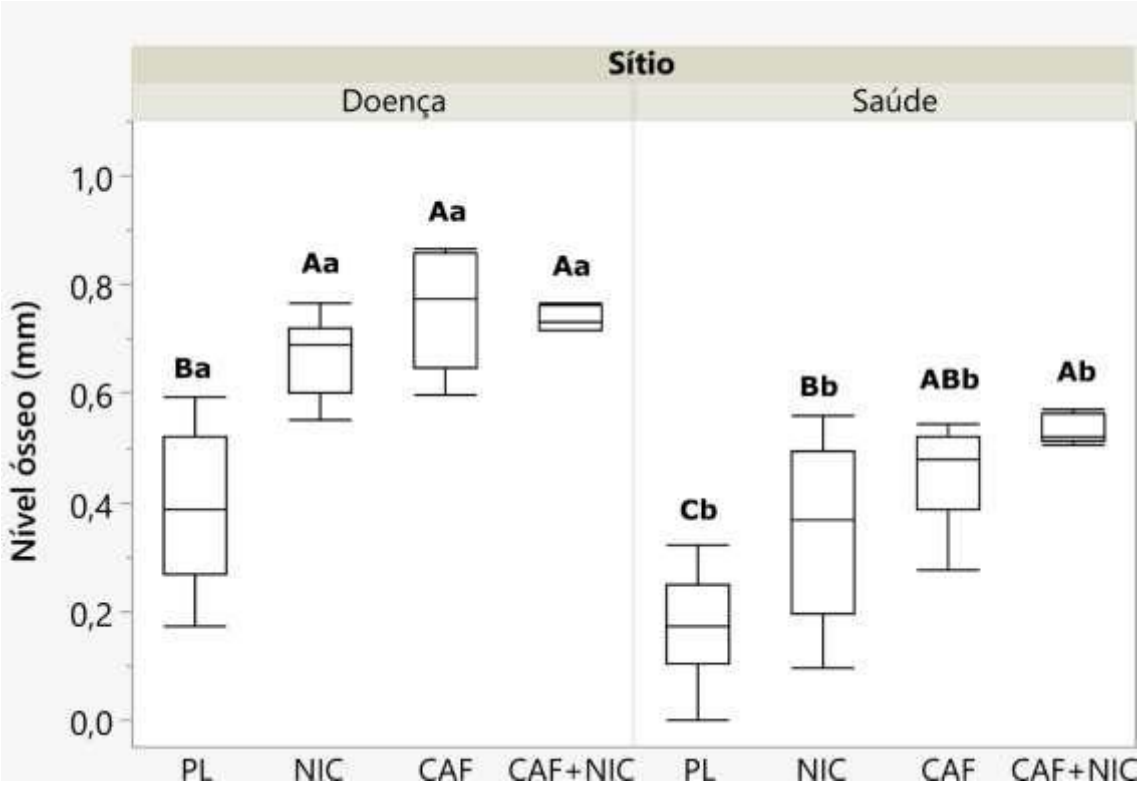
Alveolar bone loss analysis

Morphometric results – linear bone loss measurement

Intra-group analysis of alveolar bone loss showed significant statistical difference with higher bone loss at ligated sites compared to non-ligated sites ($p<0.05$), proving the model efficacy.

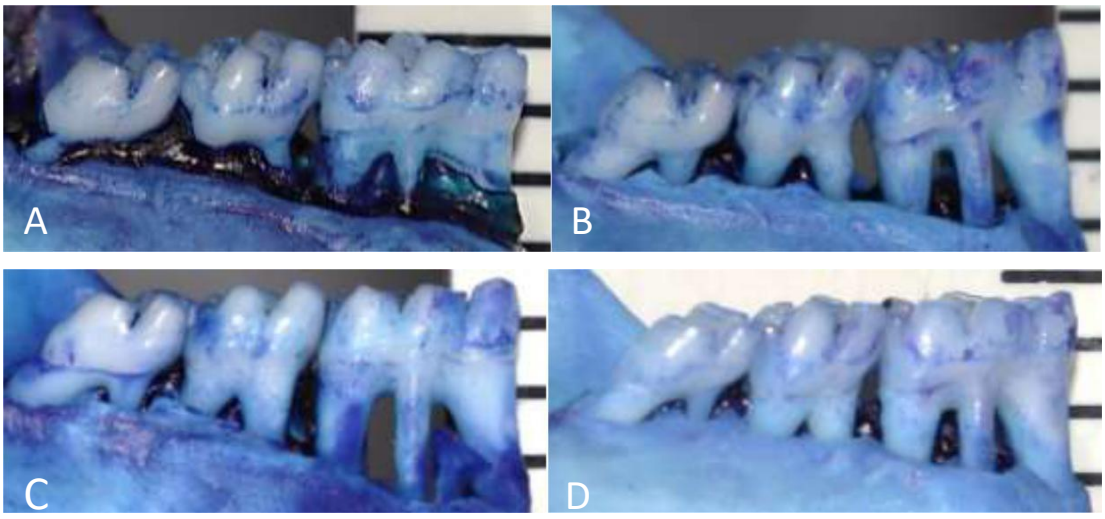
At the ligated side, NIC, CAF and CA+NIC groups presented higher bone loss compared to PL group ($p<0.05$). Higher alveolar bone loss was observed in CAF+NIC group compared to NIC and PL ($P<0.05$), while CAF/NIC isolated had higher bone loss compared to PL ($p<0.05$). The results of linear bone loss are illustrated at Figure 3.

Figure 3. Linear alveolar bone loss (millimeters) for ligated and non-ligated teeth among the groups.



Data are means (SD). Different letters indicate statistical significance. Uppercase letter compares difference between groups (Anova two-way/ Tukey Test; $p<0.05$); Lowercase letters compare difference within same group (Anova two-way/ Tukey Test; $p<0.05$).

Figure 4. Representative photographs illustrating the morphometric findings of the groups.



A - Group PL (N=10); B - Group NIC (N=10); C - Group CAF; D - Group CAF + NIC (N=10).

DISCUSSION

The ingestion of high doses of caffeine exerts negative effect on bone tissue in the progression of experimental periodontitis, in the bone repair and bone metabolism (Duarte eta l., 2009, Macedo et al., 2015, Cooper et al, 1992; Chen & Whitford, 1999; Rapuri et al, 2001; Huang et al, 2002; Tsuang et al., 2006; Rapuri et al., 2007). Smoking is a risk factor for periodontitis, with the impairment of immune-inflammatory response (Meulman et al. 2014, Loos et al., 2004, Barbieri et al. 2011). Usually, smokers also consume more caffeine due to increased caffeine metabolism caused by smoking (Brice & Smith, 2006; Istvan & Matarazzo, 1984, Swanson et al, 2009; Gurpegui et al., 2004, Reich et al., 2008; Emurian et al., 1982, Bjørngaard et al., 2017, Brown et al., 1988, Swanson et al., 1997). The present study evaluated the effect of caffein, nicotine and its combination in the progression of experimental periodontitis in rats. The results showed lower alveolar bone loss in PL group compared to the other groups, on ligated side. Higher bone loss was observed in the CAF+NIC group, when compared to the placebo group and the NIC group, while CAF had higher bone loss compared to PL on the non-ligated side. CAF+NIC group did not gain weight over time when compared to the other groups.

In the present study, NIC group presented higher bone loss on ligated side compared to PL group. This result is line with previous studies that also show higher bone loss with nicotine administration in a dose-dependent manner (Gualberto et al. 2016, Liu et al. 2010, Nociti et al.,2001, Nociti et al., 2000). Interestingly, Nociti et al., (2001, 2000) observed higher bone loss with nicotine treatment even using smaller doses than the one used in this study (0.37 mg/kg, 0.57 mg/kg, 0.73 mg/kg X 1.67 mg/kg). Remarkably, NIC also presented higher bone loss on the non-ligated side compared to PL. This result is in accordance with previous studies that also verified the negative effect of nicotine in health sites, including microstructure deteriorations in the trabecula of alveolar bone (Nociti et al.2001, Liu et al., 2010).

It is already known that nicotine modulates the progression of periodontitis with the increase in pro-inflammatory markers and influence in bone metabolism. Nicotine has been shown to dose-dependently increase the expression of cyclooxygenase 2 (COX-2) in human gingival and periodontal ligament fibroblasts (Kirschneck et al., 2015, Chang et al., 2003). Besides, studies have shown that prostaglandin E2 enhances the expression of pro-inflammatory cytokines by fibroblasts (IL-1β, IL-6 and IL-8) (Czuszak et al., 1996, Cho et al., 2014). Although this study did not analyze concentration levels of such markers, it can be

suggested that important pro-inflammatory cytokines could have their levels elevated with nicotine treatment, while anti-inflammatory markers could have been reduced.

Another important find is the higher bone loss observed when CAF was administered both alone or in association with NIC. Caffein is a substance highly consumed worldwide in beverage, in foods and drugs (Heckman et al., 2010). Our result is in line with Bezerra's et al., study (2008) who found higher bone loss with caffein treatment. On the other hand, these authors did not observed the negative effect of caffeine in the non-ligated side. It is important to observe that the caffeine dose used in the present study is three times higher than that used by Bezerra et al. The impact of caffeine on bone tissue was also studied. Duarte et al., (2009) observed lower bone formation and density in critical-size surgical defect in rat tibia. Besides, important aspects of bone metabolism are altered due to caffein treatment (bone cell viability, histomorphometry, and bone mineral index) (Cooper et al, 1992; Chen & Whitford, 1999; Rapuri et al, 2001; Huang et al, 2002; Tsuang et al., 2006; Rapuri et al., 2007).

It was already demonstrated that the association of high or low concentrations of caffeine and PGE2 clearly inhibited the proliferation of osteoblast-like cells in vitro (Kamagata-Kiyoura et al.1999). Caffeine inhibits the proliferation of osteoblast like cells and has deleterious effects on the viability of osteoblasts, increasing apoptosis of these cells (Tsuang et al., 2006).

In face of these facts, it can be hypothesized that the association of both substances can act in a synergistic way enhancing the negative effect on bone tissue and in the immune-inflammatory response, once it was observed higher bone loos in this group even in the non-ligated side compared to PL and NIC alone. Interestingly, in the non-ligated side, caffeine alone had higher values of bone loss compared to PL and was similar to the associated group. This could reinforce the hypothesis above suggested, even without the investigation of inflammatory and bone markers.

Future studies should include additional analysis to elucidate some molecular data about the immune-inflammatory cascade and in the bone metabolism process during EP in the presence of the administered substances.

Considering the limits of the present study, it can be concluded that both nicotine and caffeine in high doses and their association increase the progression of experimental periodontitis. The association of caffeine and nicotine has a destructive effect on bone tissue even in the absence of experimental periodontitis, as well as on weight gain in animals.

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3 CONCLUSÃO GERAL

Dentro dos limites do presente estudo, pode-se concluir que tanto a nicotina como a cafeína em altas doses, como sua associação aumentam a progressão da periodontite experimental. A associação da cafeína e nicotina tem um efeito destrutivo no tecido ósseo, mesmo na ausência da periodontite experimental, assim como sobre o ganho de peso dos animais.

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