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THE EFFECT OF ALCOHOL CONSUMPTION ON PERIODONTAL BONE SUPPORT IN EXPERIMENTAL PERIODONTITIS IN RATS

EFEITO DO CONSUMO DE ÁLCOOL NO SUPORTE ÓSSEO PERIODONTAL EM PERIODONTITE EXPERIMENTAL EM RATOS

Daniela Martins de SOUZA[1], **Lucilene Hernandes RICARDO**[2], **Marcela de Almeida PRADO**[3], **Fernanda de Almeida PRADO**[4], **Rosilene Fernandes da ROCHA**[5]

1- DDS, MSc, PhD Professor, Discipline of Periodontology, Faculty of Pindamonhangaba - Pindamonhangaba - Brazil.
2- DDS, MSc, PhD Professor, University of Taubaté - Taubaté - Brazil.
3- Graduate student, São Paulo State University - São José dos Campos, Dental School - Brazil.
4- Graduate student, São Paulo State University - São José dos Campos, Dental School - Brazil.
5- DDS, MSc, PhD Professor, Discipline of Pharmacology, São Paulo State University - São José dos Campos, Dental School - Brazil.

Corresponding address: Daniela Martins de Souza - Department of Bioscience and Oral Diagnosis - Av. Engenheiro Francisco José Longo, 777 Phone: (12) 3947-9034 - CEP: 12245-000 - Jardim São Dimas São José dos Campos - SP - e-mail: danimart.voy@terra.com.br

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ABSTRACT

Objective: The aim of this study was to evaluate the effect of the alcohol consumption on the periodontal bone support (PBS) in experimental periodontitis in rats. Materials and Methods: Sixty-three male rats were divided into seven groups: G1 (control); G2 (10% ethanol); G3 (nutritional control of G2); G4 (20% ethanol); G5 (nutritional control of G4); G6 (30% ethanol) and G7 (nutritional control of G6). The groups G3, G5 and G7 received controlled diets with equivalent caloric amounts to those consumed in G2, G4 and G6 respectively, with the ethanol replaced by sucrose. After anesthesia, ligatures were installed around the mandibular first molar, leaving the contralateral teeth unligated. After 8 weeks, the rats were killed and their mandibles were radiographed to measure the percentage of PBS on the distal aspect. Results: The intragroup analyses showed that presence of ligatures induced periodontitis ($p<0.05$). Unligated groups did not show significant differences among the percentages of PBS ($p=0.1969$). However, in ligated groups the rats that received alcohol (G2:48.71%±3.88; G4:47.66%±2.54; G6:47.32%±3.24) and the nutritional control group associated with a high concentration of ethanol (G7:47.40%±3.24) presented a significantly lower percentage of PBS than the other groups (G1:52.40%±2.75; G3:52.83%±2.41; G5:50.85%±4.14). Conclusions: These results demonstrated that alcohol consumption in rats may result in a direct effect on alveolar bone loss and increased development of periodontitis. In addition, they suggest that heavy caloric consumption of ethanol may also present an indirect effect on periodontal tissue as a consequence of malnutrition.
Uniterms: Ethanol; Alveolar bone loss; X-ray.

RESUMO

Objetivo: O propósito deste estudo foi avaliar o efeito do consumo de álcool sobre o suporte ósseo periodontal (SOP) em periodontite induzida por ligaduras em ratos. Materiais e Métodos: Sessenta e três ratos machos foram divididos em sete grupos: G1 (controle); G2 (álcool a 10%); G3 (controle nutricional de G2); G4 (álcool a 20%); G5 (controle nutricional de G4); G6 (álcool a 30%); G7 (controle nutricional de G6). Os grupos G3, G5 e G7 receberam dietas controladas com a mesma quantidade de calorias consumidas por G2, G4 e G6, respectivamente, com o etanol substituído por sacarose. Após anestesia foram instaladas ligaduras no primeiro molar inferior direito, permanecendo o dente contralateral sem ligadura. Após oito semanas, os ratos foram sacrificados e as mandíbulas radiografadas para medição do SOP na proximal distal. Resultados: A análise intragrupo mostrou que a presença da ligadura foi capaz de induzir periodontite ($p<0,05$). Os grupos sem ligadura não demonstraram diferenças significativas ($p=0,1969$) no SOP. Contudo, nos grupos com ligadura os ratos que receberam álcool (G2:48,71%±3,88; G4:47,66%±2,54; G6:47,32%±3,24) e o controle nutricional associado a alta concentração de etanol (G7:47,40%±3,24) apresentaram percentual de SOP estatisticamente inferior aos outros grupos (G1:52,40%±2,75; G3:52,83%±2,41; G5:50,85%±4,14). Conclusões: Os resultados demonstraram que o consumo de álcool em ratos pode resultar em efeito direto na perda óssea alveolar, aumentando o desenvolvimento da periodontite. Além disso, sugere-se que o consumo calórico pesado de etanol também pode apresentar efeito indireto nos tecidos periodontais, como conseqüência da má nutrição.
Unitermos: Etanol; Perda óssea alveolar; Raios X.

INTRODUCTION

Periodontitis is an infectious disease in which a limited number of specific bacteria and host factors act as the disease initiators and are the major determinants of disease occurrence and severity[24]. Periodontitis involves the destruction of supporting structures of teeth including the periodontal ligament, alveolar bone and gingival tissues[13]. There is a large variety of factors that influence the progression of periodontitis, including individual characteristics, social and behavioral factors, systemic factors, genetic factors, tooth factors, and microbial composition of the dental biofilm[6,22,26]. The social and behavioral factors include cigarette smoking, socioeconomic status, nutritional status, psychological factors and excessive alcohol consumption[22].

Studies that have evaluated the effects of alcoholism on oral tissues suggest that it may be associated with a greater risk for development of periodontal problems due to poor oral hygiene[11,16,21]. However, there is evidence that persistent alcohol abuse affects the severity of periodontal disease, when blood levels of gamma-glutamyl transpeptidase (GGTP), a liver enzyme indicator of alcohol consumption, were determined[12]. A population study that used self-reported questionnaire regarding alcohol consumption suggested a significant relationship among alcohol, gingival inflammation and clinical attachment loss, after controls to account for major confounders[27]. Recently, a moderate but consistent dose-dependent relationship between alcohol consumption and increased severity of clinical attachment loss in periodontal disease was found[28]. Considering that no information is available from an "in vivo" model to test the hypothesis that alcohol consumption enhances bone loss in ligature-induced periodontitis, the aim of this study was to evaluate the effect of alcohol consumption on the periodontal bone support (PBS) in experimental periodontitis in rats.

MATERIALS AND METHODS

Animals

Sixty-three adult male Wistar rats (4 months of age) weighing 460g in average were used in the study. All rats were housed under similar conditions and received specific diets according to the experimental group. The Institutional Animal Research Committee at the University of São José dos Campos (São Paulo, Brazil) approved the protocol.

Experimental groups

The rats were randomly distributed into seven groups of nine animals each. Group G1: control (normal rat chow and water); G2: 10% ethanol (normal rat chow and liquid diet containing 10% v/v ethanol); G3: nutritional control of G2 (rat chow and liquid diet containing sucrose solution, diet isocaloric to G2); G4: 20% ethanol (normal rat chow and liquid diet containing 20% v/v ethanol); G5: nutritional control of G4 (rat chow and liquid diet containing sucrose solution, diet isocaloric to G4); G6: 30% ethanol (normal rat chow and liquid diet containing 30% v/v ethanol) and G7: nutritional control of G6 (rat chow and liquid diet containing sucrose solution, diet isocaloric to G6).

The objective of the nutritional control groups was to verify the occurrence of malnutrition associated with alcoholism (indirect effect of alcohol). The groups G3, G5 and G7 received controlled diets (normal rat chow and liquid diet containing sucrose solution) with equivalent caloric amounts to those consumed in G2, G4 and G6 respectively.

Experimental procedures

The animals from G2, G4 and G6 groups were submitted to an adaptation period in which the ethanol concentration was increased until it reached experimental concentrations. A solution with 5% v/v ethanol was administered to G2 group for 7 days, group G4 received 5% and 10% v/v ethanol for 7 days each and in group G6 5%, 10% and 20% v/v ethanol was administered for 7 days each. Finally the rats completed the remaining 8 weeks with a diet containing experimental concentrations. One day after the ethanol administration, the nutritional control animals were fed an equal amount of normal rat chow as consumed by the associated alcohol groups and an equal volume of liquid diet, with the ethanol replaced by isocaloric amounts of sucrose. During the experimental period the diet consumed was evaluated and expressed descriptively.

After the adaptation period, general anesthesia was induced by intramuscular administration with solution of 13mg/Kg of 2% xylazine hydrochloride (Rompum-Bayer-São Paulo, SP, Brazil) and 33mg/Kg of ketamine (Francotar-Virbac-Roseira, SP, Brazil). To induce periodontitis, one of the mandibular first molars of each rat was randomly assigned to receive a cotton ligature around the cervical region. The ligature was knotted on the mesial side of the tooth. The contralateral tooth was left unligated to serve as control.

After 8 weeks, the rats were killed and their mandibles were carefully removed and fixed in 10% neutral formalin for 48h. The defleshed alveolar process with the three molars was dissected from each side of the mandible.

Radiographic analysis

Each specimen was oriented to the buccal and lingual cusps of first and second molars were superimposed and splinted on the sensor by a plastic slab. Digital radiographs were obtained using a RVG® (RadioVisioGraphy-Trophy radiology inc.-Marietta/USA) computer imaging system. Electronic sensors were exposed at 65 Kv and 7 mA for 0.08s and the source-to-sensor distance was always 30cm.

To obtain sufficient reproducibility of the alignment on the image, two criteria had to be fulfilled: the teeth should not overlap each other interproximally and the buccal cusp tip of first and second molars should be superimposed to the corresponding lingual cusp tip.

Periodontal bone support (PBS) was measured on radiograph images with Image Tool v.3.0 (UTHSCSA). All measurements were made at the distal aspect of the

mandibular first molars by the same blind examiner. Three points were taken as references: the apex of the distal root (A), the distal cusp tip (C) and the bottom of the deepest bony defect distal to the tooth (B). Afterwards the distances apex-cusp tip (AC) and apex-deepest bone defect (AB) were measured in mm. Periodontal bone support was determined by the formula: PBS=AB/AC x100[3-14] (Figure1). All measurements were made without knowledge of the group to which the rat belonged.

The measurements were performed three times on each side and the mean record was considered for the PBS. A higher value of PBS indicates lower periodontal bone destruction. Periodontal bone support is an assessment of remaining periodontal bone (mm).

Reproducibility

Before statistical analysis was performed, the examiner was trained by double measurements of 54 specimens (40%), with a one-week interval. Paired *t* test statistics was run and no differences were observed in the mean values for comparison (p=0.8569). Additionally, Pearson's correlation coefficient was obtained between the 2 measurements and revealed a very high correlation (0.99, p = 0.000).

Statistical analysis

Data were expressed as mean values and standard deviation of perceptual (%) of remaining periodontal bone (mm). The paired *t*-test (α=0.05) was used for intragroup comparisons between ligated and unligated teeth. One-way analysis of variance (ANOVA) (α=0.05) and the Tukey test for subsequent multiple comparisons (α=0.05) were used to determine significant differences in periodontal bone support among the treatment groups.

RESULTS

The 10% alcohol group (G2) and its nutritional control group (G3) consumed an average of 27.20 kcal/day provided by the liquid diet and 99.14 kcal/d provided by normal rat chow. Ethanol represented 21.72% of the total dietary energy intake. The 20% alcohol group (G4) and its nutritional control group (G5) consumed an average of 48.44 kcal/d provided by the liquid diet and 85.14 kcal/d provided by normal rat chow, corresponding to 36.55% ethanol-derived calories. The 30% alcohol group (G6) and its nutritional control (G7) consumed an average of 43.41 kcal/d provided by the liquid diet and 52.77 kcal/d provided by normal rat chow. Alcohol represented 45.21% of the total dietary energy intake (Table 1).

Radiographic analysis showed that cotton ligatures placed around the teeth promoted periodontitis, determined by lower percentages of periodontal bone support in ligated

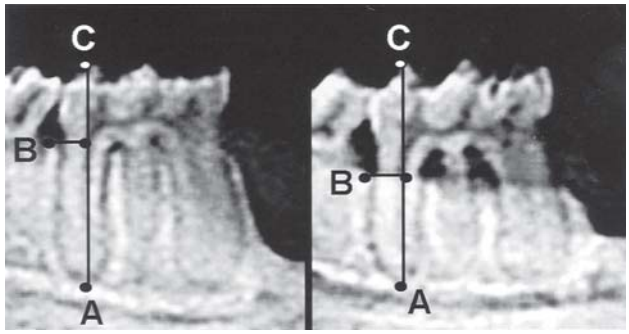

FIGURE1- Periodontal bone support (PBS) on the distal side of unligated (right) and ligated (left) teeth. AB indicates the distance from apex (A) to deepest bony defect (intersection of two lines), measured in mm. AC indicates the distance from apex (A) to cusp tip (C) measured in mm. PBS is calculated according to the formula: PBS=AB/AC x 100%

TABLE 1- Mean and standard deviation of dietary calories and caloric percentage of liquid and solid diet

Group	Alcohol in liquid diet (kcal/d)	Rat chow (kcal/d)	Total diet (kcal/d)	% calories liquid diet	% calories solid diet
G1	0.00 (± 22.16)	112.82 (± 22.03)	112.82	0	100
G2 G3	27.20 (±1.08)	99.14 (±10.13)	126.34 (±10.37)	21.72	78.28
G4 G5	48.44 (±4.12)	85.14 (±12.81)	133.58 (±14.92)	36.55	63.45
G6 G7	43.41 (±5.50)	52.77 (± 6.74)	96.18 (±9.94)	45.21	54.79

teeth than in unligated teeth ($p< 0.05$). In unligated teeth, intergroup analysis did not show significant differences among PBS values ($p>0.05$). However, when periodontitis was induced, analysis revealed greater periodontal bone destruction ($p<0.05$) in the rats receiving alcohol, observed by a lower percentage of PBS in the 10% alcohol (G2), 20% alcohol (G4) and 30% alcohol (G6) groups, than in the rats receiving water (G1) and isocaloric sucrose solution (G3 and G5). A dose-dependent response was not observed among the alcohol groups ($p> 0.05$). Moreover, alcohol may have an indirect effect at high doses on the percentage of the remaining bone support, as observed in the isocaloric group G7 (Table 2).

DISCUSSION

The present study was designed to evaluate the effect of alcohol consumption on periodontal bone support in ligature-induced periodontitis in rats. The results demonstrated that this rat model is capable to evaluate the association between alcohol and periodontal disease.

The experimental period of alcohol consumption was based on a previous study that showed the negative effects of ethanol on bone metabolism in 8 weeks[8,9] or in more reduced period[5,7,8] associated with a modified liquid diet containing 8.1% v/v ethanol, corresponding to 35% ethanol-derived calories.

The ligature-induced bone loss was particularly visible on day fifteen. Subsequently, stagnation in the progression of bone loss up to day sixty was observed, indicating a diminished effect of the ligatures[15]. In the present study, the ligature-induced periodontitis lasted for fifty-six days, because it was associated with the total experimental period of ethanol administration. A similar time period was used by Duarte et al.[4] (2004) who killed the rats sixty days after ligature placement to evaluate the impact of an estrogen-deficient state and its therapies on periodontal tissues.

TABLE 2- Mean and standard deviation of PBS (%) in ligated and unligated teeth

Group	Unligated	Ligated
G1	63.66 ± 1.80 ^{aA}	52.40 ± 2.75 ^{bA}
G2	61.50 ± 2.51 ^{aA}	48.71 ± 3.88 ^{bB}
G3	63.29 ± 2.34 ^{aA}	52.83 ± 2.41 ^{bA}
G4	62.20 ± 1.77 ^{aA}	47.66 ± 2.54 ^{bB}
G5	61.54 ± 2.12 ^{aA}	50.85 ± 4.14 ^{bA}
G6	62.29 ± 1.87 ^{aA}	47.32 ± 5.11 ^{bB}
G7	62.75 ± 1.60 ^{aA}	47.40 ± 3.24 ^{bB}

Capital letters should be considered in columns (intergroup analysis: ANOVA, p < 0.05). Lower case letters in lines (intragroup analysis: paired t-test, p < 0.05). Means followed by different letters differ statistically.

The present study found a relationship between alcohol consumption and periodontal bone support reduction following the experimental induction of periodontitis in rats. The effects of alcohol on periodontal disease exist and this relationship has been explained by biological plausibility through different mechanisms. Chronic alcoholic patients show an increased risk for developing severe infection, which may be due to altered immune response[19,25]. Alcohol has a toxic effect on the liver causing a negative effect on the clotting mechanism[18]. People who are classified as heavy alcohol drinkers frequently present nutritional disorders resulting from protein and vitamin deficiency[17,18]. Additionally, ethanol alters the bone metabolism demonstrated by the dramatic effects on bone in rats[7,8,9]. As a consequence of the toxic effects on the liver, bone, immune system and nutrition, alcohol may interfere with the mechanism of inflammatory response in periodontal disease.

Previous studies in humans have shown that alcohol consumption is associated with increased severity of periodontal disease[12,27,28]. However, this is one of the first studies to show a relationship between periodontal supporting bone in experimental rat periodontitis and alcohol consumption.

The structure of the periodontal tissue in rats is very similar to that of humans[23]. Several methods have been applied to assess periodontal bone level in rats: morphometric analysis on defleshed jaws[3,15], histometric evaluation in the furcation region[1,2] and radiographic analysis[3,10,20]. However, the radiographic method was used for the detection of intrabony interproximal defects[14] and was also used in this study.

The present study used different alcohol concentrations, corresponding to increasing ethanol-derived calories, and a radiographic method for the progressive evaluation of periodontal bone loss. The results were positive in relation to alcohol consumption and increased disease progression, although no evidence was found of a dose-dependent relationship. The lack of an ethanol dose-dependent relationship between alcohol consumption and periodontal disease revealed in this study could be explained by the assessment method used to detect minimal differences or by the short experimental period in which the alterations occurred. Further studies are required to confirm this interaction, because a dose-dependent relationship appears to be consistent with biological plausibility.

Ethanol is a psychoactive drug and has a substantial energy value (7.1 Kcal/g). In the heavy drinker, alcohol represents 50% of the total dietary energy intake, in average. As a consequence, alcohol displaces many normal dietary nutrients resulting in primary malnutrition. Moreover, secondary malnutrition may result from maldigestion or malabsorption caused by gastrointestinal complications associated with alcoholism[17]. In the present study, alcohol represented 45.21% of the total dietary energy intake in the 30% alcohol group and its nutritional control (G6 and G7, respectively). In group G7, the occurrence of nutritional alteration may demonstrate the indirect effect of ethanol on increased alveolar bone loss when ethanol was replaced by

an isocaloric amount of sucrose. This result suggests that high doses of alcohol may be associated with the development of malnutrition in rats and can increase periodontal bone loss caused by periodontitis.

CONCLUSION

In conclusion, the present study demonstrated that the direct effect of alcohol consumption modulates the progression of periodontal bone loss resulting from experimental periodontitis. In addition, alcohol may have an indirect effect on increased bone destruction in periodontal disease in rats associated with heavy caloric consumption. These results indicate that alcohol intake may be a risk indicator for periodontitis but further studies are required to confirm this interaction.

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