Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

Effects of *Cachaça*, a typical Brazilian alcoholic beverage, on alveolar bone loss and density: A study in peripubertal rats

Marta Ferreira Bastos [a,*], *Giovanna Lima Dore Gaag* [a], *José Renato Romero* [b], *Joel José Megale Gabrili* [a], *Marcelo Rocha Marques* [c], *Poliana Mendes Duarte* [a]

[a] *Department of Periodontics, Dental Research Division, Guarulhos University, Guarulhos, São Paulo, Brazil*
[b] *Department of Physiotherapy, Guarulhos University, Guarulhos, São Paulo, Brazil*
[c] *Department of Morphology, Division of Histology, School of Dentistry at Piracicaba, University of Campinas, Piracicaba, São Paulo, Brazil*

ARTICLE INFO

Article history:
Accepted 9 October 2013

Keywords:
Ethanol
Alveolar bone loss
Ligature
Tartrate-resistant acid phosphatase
RANK ligand
Osteoprotegerin

ABSTRACT

Objective: The aim of the present study was to assess the impact of chronic consumption of *Cachaça* on alveolar bone loss (BL) induced by ligature and on alveolar bone density (BD) in peripubertal rats.

Design: Male Wistar rats were assigned into one of the following groups: *Control*: non-ingestion of *Cachaça* ($n = 15$); *Cachaça:* ingestion of ascending concentrations of *Cachaça* during 100 days ($n = 15$). 70th day after the beginning of *Cachaça* ingestion, one first mandibular molar received a ligature while the contralateral tooth was left unligated. After 30 days, the rats were killed. BL, BD, the positive cells for tartrate-resistant acid phosphatase (TRAP), receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) were analyzed in the furcation area of the ligated and unligated mandibular molars.

Results: The *Cachaça* group presented greater BL ($0.75 \pm 0.1\ mm^2$ for *Cachaça* and $0.66 \pm 0.1\ mm^2$ for control group, respectively) and number of RANKL and OPG+ cells and lower BD ($60.3 \pm 4.2\%$ for *Cachaça* and $76.8 \pm 3.8\%$ for control group, respectively) and number of TRAP+ cells around ligated teeth ($p < 0.05$), when compared to the control group. The *Cachaça* group ($0.42 \pm 0.02\ mm^2$) also presented a higher BL around unligated teeth when compared to control group ($0.31 \pm 0.05\ mm^2$).

Conclusions: *Cachaça* consumption *per se* and in the presence of ligature negatively affects alveolar bone by increasing the alveolar BL and reducing BD.

1. Introduction

The World Health Organization (WHO) estimates that approximately 6.13 L of pure alcohol were consumed per person in 2005.[1] Moreover, 4% of all deaths in world were attributed to heavy alcohol consumption, which was especially fatal for males aged 15–59 years.[1] In addition, epidemiological data indicates that high rate of adolescents aged between 12 and 20 years are drinkers and, had drunk alcohol for the first time before the age of 13 years.[2]

* *Corresponding author at*: Praça Tereza Cristina, n. 229, Centro–Guarulhos, CEP: 07.023-070, SP, São Paulo, Brazil. Tel.: +55 11 24641758; fax: +55 11 24641758.
E-mail address: mfbastos@prof.ung.br (M.F. Bastos).
0003–9969/$ – see front matter
http://dx.doi.org/10.1016/j.archoralbio.2013.10.002

In Brazil, 31.8% of the population are considered former drinkers, whereas 11.2% of the population are alcohol dependent.[3] Liver cirrhosis and traffic accidents are the main causes of alcohol-associated deaths in this country.[3] *Cachaça* is a typical Brazilian alcoholic beverage produced from fermentation and distillation of fresh sugarcane juice, which possesses an high alcoholic content that usually varies from 38 to 48% (v/v) at 20 °C.[4,5]

Long-term alcohol consumption has been associated with an increased risk of oral diseases such as caries and periodontitis.[6] Clinical studies regarding the relationship between periodontitis and alcohol consumption presented contradictory results. While some studies have demonstrated a positive correlation between alcohol and periodontal diseases,[7–11] others have shown no or a weak association between both conditions.[12,13] Some investigations have suggested that the association between alcohol and periodontal diseases is attributed to the disability of alcoholics to practice an adequate oral hygiene.[14,15] However, Amaral et al.[16] demonstrated negative effects of alcohol consumption on important periodontal parameters, such as clinical attachment level and probing depth even in the presence of an adequate plaque control.

In rats, studies have suggested that alcohol consumption may increase the alveolar bone loss[17,18] and the expression of inflammatory mediators (*e.g.* inducible nitric oxide synthase) in gingival tissues in a ligature-induced periodontitis model.[19,20] Nevertheless, Liberman et al.[21] concluded that the intake of low concentrations of alcohol did not affect the alveolar bone loss in this experimental model. However, animal studies differ regarding the type of alcoholic beverage, dose of ethanol, time and *via* of alcohol administration and animal age. Although the ethanol effects upon periodontium have been evaluated by several studies, no study has evaluated the effects of *Cachaça* on the periodontal tissues of peripubertal rats. Therefore, the aim of the present study was to assess the impact of chronic consumption of *Cachaça* on alveolar bone loss (BL), induced by ligature, and on alveolar bone density (BD) in peripubertal rats. It was hypothesized that the chronic consumption of *Cachaça* would promote the greatest level of alveolar BL and the lowest percentage of alveolar BD.

2. Materials and methods

2.1. Animals

Thirty male Wistar rats presenting 50 days of age, acquired from the Butantan Institute (São Paulo, São Paulo, Brazil), were used in this study. The rats weighed 182.83 ± 4.49 g at the beginning of the experiments. During the entire experimental period (100 days), each animal was housed alone in a plastic cage in the Bioscience Laboratory of Guarulhos University in a room with a 12-h light/dark cycle and temperature of between 22 and 25 °C. The animals were maintained with access to food *ad libitum* (Labina, Purina®, Paulinia, SP, Brazil). The Institutional Committee for Animal Care and Use at São Paulo University (São Paulo, São Paulo, Brazil) approved the study protocol (2476/2011).

2.2. Alcohol consumption and experimental groups

After the acclimatization period (5 days), the rats were randomly assigned to one of the following groups: control ($n = 15$): animals without *Cachaça* ingestion; *Cachaça* ($n = 15$): animals subjected to ingestion of *Cachaça*. The *Cachaça* group consumed *Cachaça ad libitum* (trademark: ''51''; Müller Beverage Company, Pirassununga, São Paulo, Brazil), while the control group consumed water *ad libitum* during 100 days.

The protocol of *Cachaça* ingestion was adapted from Pereira et al.[22] Briefly, in the 50 initial days, the animals ingested increasing doses of *Cachaça* (10% (v/v) and 15% (v/v) for 15 days/concentration; 20% (v/v) and 25% (v/v) for 10 days/concentration). Subsequently, the animals ingested 30% (v/v) of *Cachaça* during the remaining 50 days.

2.3. Ligature placement

Seventy days after the beginning of the *Cachaça* consumption, the animals were anesthetized by intraperitoneal administration of xylazine (0.3 mL/kg of weight, Virbaxil; Virbac Brazil Industry and Trade, Roseira, SP, Brazil) and ketamine (0.5 mL/kg of weight, Francotar; Virbac Brazil Industry and Trade, Roseira, SP, Brazil) for ligature placement. A cotton thread was placed around the first right mandibular molar, in a cervical position, in order to induce alveolar bone loss, while the contralateral molar remained without a ligature for use as a control. Thirty days after the ligature placement the animals were killed by CO_2 inhalation. As such, the animals ingested *Cachaça* during 70 days before the ligature placement and during 30 days after ligature permanence.

2.4. Examiner calibration

For all analysis (histometry and stained cell counts), calibration exercises were conducted before the beginning of the study. Intra-examiner calibration was achieved by examining ten non-study sections twice, with an interval of 48 h between measurements. Intra-examiner reproducibility of all measurements achieved ≥90% by Intra-class correlation.

2.5. Histological procedures and histometric analysis

Bone loss (BL) and bone density (BD) measurements were performed in ligated and unligated mandibular first molars. Rats' lower jaws were removed and fixed in formalin buffer for 12 h. Subsequently, the specimens were decalcified in a solution containing 4.13% ethylene-diamine tetraacetic acid for 60 days, dehydrated in an ascending series of ethanol solutions, cleared in xylene and embedded in paraffin. Serial sections (5 μm) were obtained in a mesio-distal vertical direction of the mandibular molars. After excluding the first and last sections, in which the furcation area was totally evident, nine sections, 30 μm apart, from each mandibular first molar were obtained. Of these nine sections, five sections were stained with haematoxylin and eosin (HE) solutions for histometric analyses while four sections were used for histochemistry and immunohistochemistry evaluations. For BL, the areas between the inter-radicular bone crest and the furcation roof (mm^2) of the mandibular molars were