Alveolar bone loss induced by chronic ethanol consumption from adolescence to adulthood in Wistar rats


1Laboratory of Functional and Structural Biology, Institute of Biological Sciences; Federal University of Pará, Belém-Pará, 66075-900.
2Laboratory Pharmacology of Inflammation and Behavior, Institute of Health Sciences, Federal University of Pará, Belém-Pará, 66075-900.
3Department of Pharmacology, Federal University of Santa Catarina, Florianópolis-Santa Catarina, 88049-900, Brazil.

Received 23 January 2014; revised 16 February 2014

Though there are literature indicating the bone loss due to alcohol consumption, studies on the association between ethanol consumption and periodontal breakdown in animals are either scarce or have provided conflicting results. Here, we investigated the effects of chronic alcohol exposure from adolescence to adulthood on the alveolar bone in rats. Wistar rats were exposed to ethanol (6.5 g/kg/day) in a solution of 22.5% (w/v) or distilled water (control) by gavage from 35 days of age (adolescent) until 90 days (adulthood). Evaluation of the bone loss was performed using scanning electronic microscopy, in which the distances between the cement-enamel junction and the alveolar bone crest from the palatal side of the first molar mandibular were measured. The measurements obtained were tabulated and analyzed using Student’s t-test. Alcohol-treated group revealed greater bone loss in comparison to the control group. These findings indicate that heavy chronic alcohol exposure from adolescent to adulthood can induce alveolar bone loss in rats associated to absence of periodontitis.

Keywords: Alcoholism, Dental, Periodontitis

Ethanol is a widely used drug of abuse among adolescents due to its easy availability and acceptance by society. Early use of ethanol is reflected in the higher incidence of alcoholism in adulthood, given that, over 80% of drinkers reported alcohol-related problems before the age of 30 and 40% between 15–19 years of age. Adolescents are recognized as a nutritionally at-risk group. About 90% of skeletal development and attainment of peak bone mass occur during this stage of life. This could be impaired in the presence of ethanol when the intake of calcium and other vitamins (D) is reduced. Further, a decrease in blood vitamin D levels induces release of parathyroid hormone, which could increase the osteoclast activity and bone resorption.

Alveolar bone is a specialized part of periodontium that forms the primary support structure for teeth. Alveolar bone develop directly from ectomesenchymal cells (intramembranous or desmal ossification), unlike bones of the trunk, which develop on the basis of a pre-existing cartilage model (endochondral ossification). Alveolar bone also differs from other bones by presenting all forms of bone histology, as a Harversian system and lamellated and bundle bone. Functionally, is subjected to continual and rapid remodeling associated with functional demands of mastication. Inflammatory periodontal disease leads to dental element loss in adults, provoked by bacterial plaque accumulation, followed by edema, inflammatory cells migration and pro-inflammatory mediators release, resulting in alveolar bone loss. Dantas et al. reported that heavy ethanol exposure produces additive effects on interleukin (IL)-1b, inducible nitric oxide synthase (iNOS) mRNA expression and iNOS activity induced by periodontitis in rats. Further, harmful effects of ethanol intake on periodontal breakdown depends on the dose and period of exposure.

Alcohol consumption is known to decrease bone mineral density, affect bone repair and bone

*Correspondence: Telefax: 00 5591 3201 7891 E-mail: rafalima@ufpa.br, rafaelrodrigueslima@hotmail.com
formation\textsuperscript{19}, and increase bone resorption, thus resulting in bone loss\textsuperscript{19}. However, studies evaluating ethanol intake and periodontal breakdown in animals are scarce. Souza et al.\textsuperscript{20} demonstrated alveolar bone loss and increased risk of periodontal disease in the ligature-induced periodontitis in adult rats following 8 weeks of ethanol consumptions (10, 20 and 30 w/v). Later, they compared low alcohol consumption (22% of total caloric value) and heavy alcohol consumption (36% of total caloric value), concluded that low consumption resulted in greater bone loss and no significant changes were observed due to heavy alcohol consumption in the ligated group when compared to the control group\textsuperscript{21}.

Another study demonstrated that 10 and 20% alcohol administration in the ligated group resulted in alveolar bone loss, while the 20% group was more effective in the alveolar bone loss than the 10% group; thus, the alveolar bone loss appeared to occur in a dose-dependent manner although no significant difference was observed in the non-ligated group\textsuperscript{22}.

Liberman et al.\textsuperscript{23} reported that 46-60 days old Wistar rats which received ethanol in low concentration (5%) systematically presented less alveolar bone loss in unligated teeth. However, no significant difference was observed between ethanol and control groups in ligated teeth\textsuperscript{23}. Further, adult male rats that received a ligature in the first lower molar and were submitted to self-administration of alcohol for successive 5% increases till 25% (for five weeks) showed increased bone destruction in periodontal disease that was intensified by co-administration of nicotine\textsuperscript{24}.

Recently, Bastos et al.\textsuperscript{25} designed a protocol in which peripubertal rats were submitted to chronic ethanol exposure (100 days) associated with molar ligature in the 70\textsuperscript{th} day and the contralateral tooth was left unligated. It was observed that ethanol exposure per se and in the presence of ligature increased alveolar bone loss and diminished bone density.

However, influence of alcohol in periodontal breakdown still remains unclear. Therefore, we investigated the effects of chronic ethanol exposure from adolescence to adulthood on the alveolar bone in rats in the absence of ligature-induced periodontitis.

**Material and Methods**

**Animals**—The 35-day-old female Wistar rats obtained from the Central Animal Facility of the Federal University of Pará (Belém, Brazil), were housed under standard conditions (25 °C, 12 h L:D cycle) with food and water available ad libitum. All experimental procedures were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985) and European Commission Directive 86/609/EEC for animal experiments under license of the Ethics Committee on Experimental Animals of the Federal University of Pará (approval number CEPAE-UFPA: BIO043-12). All possible efforts were made to avoid animals suffering and distress.

**Experimental design**—Twenty animals (n = 10 per group) were randomly assigned to receive distilled water or alcohol 6.5 g/kg/day (22.5% w/v) through orogastric cannula over a period of 55 days (i.e., from 35\textsuperscript{th} to 90\textsuperscript{th} day of life) according to the procedure described previously\textsuperscript{26}. Considering that gavage process presents stressful stimulus, control group also received the same procedure with administration of water in order to standardize the conditions to both groups and avoid factors that could influence the results. In order to investigate putative effects of ethanol intoxication on overall poor nutrition levels that may directly affect the bone development, the animals’ body weight was controlled during the entire period of drug administration, offering the same amount of food for both groups. After chronic ethanol administration, the animals were anesthetized, perfused with 0.9% heparinized saline solution and 4% paraformaldehyde, and then their jaws were dissected.

**Measurement of alveolar bone loss**—The hemimandibles of each group (n = 10 per group) were examined with a scanning electron microscope (SEM). The samples were cleaned with 1% sodium hypochlorite (NaOCl) for 5 min, immersed in 17% ethylenediaminetetraacetic acid (EDTA) for 10 s, and washed with distilled water in ultrasonic bath for 30 s. Then they were dried at room temperature, prepared, and examined using an SEM (LEO-1430, ZEISS, Germany). Using the SEM images, the distances between the cement-enamel junction and the alveolar bone crest from the palatal side of the first molar were measured\textsuperscript{27}.

**Statistical analysis**—The values of animals’ body weight and distances between the cement-enamel junction and the alveolar bone crest are expressed as mean ± SD (n = 10 per group). Statistical comparison between the two groups was performed by two-way ANOVA, followed by Bonferroni’s test for body
weight and Student’s *t*-test for distances between the cement-enamel junction and the alveolar bone crest using the software GraphPad Prism 5.0 (GraphPad Software, Inc., USA). The accepted level of significance was *P* < 0.05.

**Results**

Chronic ethanol intake from adolescence to adulthood on body weight of female Wistar rats were evaluated each six days, since first day of administration (D1), till the last day (D10). Ethanol group present the same values of body weight until before 60 days old (30 days of administration). However, at the adulthood, the rats showed reduced body weight gain during the study period (Fig. 1).

Representative photomicrographs of hemi-mandibles from the groups exposed to distilled water or alcohol from adolescence to adulthood are presented in Figs. 2A and 2B, respectively. The distance measured between the cement-enamel junction and the alveolar bone crest used as indicative of alveolar bone loss is presented in Fig. 2C. Statistical analysis showed significantly higher bone loss (*P* < 0.0001) in the alcohol-treated group (1.070 ± 0.0129) compared to the control-treated group (0.9331 ± 0.0126) that received distilled water.

**Discussion**

The present findings demonstrate that heavy chronic ethanol intake from early adolescence to adulthood can induce alveolar bone loss in rats in the absence of any other factor inducing periodontal disease. In addition, the observed alveolar bone loss is associated to reduced gain of weight that could contribute to reinforce the chronic ethanol intoxication effects, however not directly alveolar bone loss. The present protocol of ethanol administration was based on pilot studies carried out with different ethanol doses (1-8 g/kg/day) and previous studies showing that ethanol intoxication (6.5 g/kg/day) during the developing CNS may induce long-lasting neurobehavioural impairments in rats. Moreover, the drinking period (from adolescence to adulthood) was chosen based on the available report that chronic ethanol consumption during this period impairs skeletal development through effects on osteoblast gene expression, bone mineral density, and bone strength.

Diabetes, smoking, oral hygiene, education, age, and gender have been demonstrated to be directly associated with periodontitis. However, non-drinkers and regular drinkers have been shown to have a higher risk of severe clinical attachment loss (CAL) as compared to occasional drinkers. Another study reported that alcohol drinkers have a higher risk of periodontitis than non-drinkers, while no significant relationship has been observed between alcohol consumption and periodontal disease.
Similar results were observed in a group of heavy drinkers who did not have periodontal disease related to alcohol consumption, although apical lesions, a greater degree of plaque, and decayed surfaces were observed, indicating that lifestyle interferes with dental health. In spite of the available clinical literature, the studies evaluating this relationship in animals are scarce. For instance, some authors have demonstrated greater while others reduced alveolar bone loss in ligated teeth in rats receiving ethanol. It must be highlighted that these previous studies have investigated the effects of voluntary ethanol intake in adult male rats during a period of 60-75 days, investigating the effects of voluntary ethanol intake in adult male rats during a period of 60-75 days, and observed, indicating that lifestyle interferes with dental health.

In conclusion, the present findings reinforce the notion of the risk of ethanol consumption during adolescence, demonstrating that chronic alcohol exposure from early adolescent to adulthood can induce alveolar bone loss in rats even in the absence of any other factor inducing periodontal disease. Thus, the exact mechanism that underlying the alveolar bone loss mediated by alcohol consumption needs to be elucidated.

Acknowledgement
We thank Research Support Foundation of the State of Pará (FAPESPA) for financial support. RDP is supported by a research fellowship from CNPq. The authors have no financial or personal conflicts of interest related to this work.

References
BANNACH et al.: CHRONIC ETHANOL INDUCES ALVEOLAR BONE LOSS