ARCHIVES OF ORAL BIOLOGY 57 (2012) 1385–1394

Available online at www.sciencedirect.com

SciVerse ScienceDirect

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

Influence of chronic alcoholism and oestrogen deficiency on the variation of stoichiometry of hydroxyapatite within alveolar bone crest of rats☆

Adriana M.P.S. Marchini [a,*], *Camila P. Deco* [a], *Karina B. Lodi* [a], *Leonardo Marchini* [a,b,c], *Ana M.E. Santo* [d,e], *Rosilene F. Rocha* [a]

[a] *Department of Biosciences and Diagnostics, São José dos Campos School of Dentistry, São Paulo State University – UNESP, São José dos Campos, São Paulo, Brazil*

[b] *School of Dentistry, University of Vale do Paraíba – UNIVAP, São José dos Campos, São Paulo, Brazil*

[c] *Department of Dentistry, University of Taubaté – UNITAU, Taubaté, São Paulo, Brazil*

[d] *Institute of Research and Development – IP&D, Laboratory of Biomedical Vibrational Spectroscopy, University of Vale do Paraíba, São José dos Campos, São Paulo, Brazil*

[e] *Department of Mathematical and Earth Sciences, Federal University of São Paulo – UNIFESP, Diadema, São Paulo, Brazil*

ARTICLE INFO

Article history:
Accepted 21 April 2012

Keywords:
Alcoholism
Ovariectomy
Periodontium
Fluorescence spectrometry
Calcium
Phosphorous

ABSTRACT

Objective: Previous findings suggest that chronic alcoholism and oestrogenic deficiency may affect bones in general (including alveolar bone) and increase individuals' susceptibility to the development of periodontal disease. The aim of this study was to assess possible alterations in the chemical composition of alveolar bone in rats subjected to chronic alcoholism, oestrogen deficiency or both.

Design: Fifty-four rats were initially divided into two groups: ovariectomized (Ovx), and Sham operated (Sham). A month after surgery, the groups were sub-divided and received the following dietary intervention for eight weeks: 20% alcohol, isocaloric diet and ad libitum diet. Samples of the mandible, in the alveolar bone crest region, were analyzed to verify possible changes in the stoichiometric composition of bone hydroxyapatite, by measuring the relationship between the concentration of calcium and phosphorus (Ca/P ratios), using micro X-ray fluorescence spectrometry.

Results: The ad libitum groups presented the highest average values of Ca/P ratios, while the groups with dietary restrictions presented the smallest average values. The Ovx ad libitum group presented the highest values of Ca/P ratios (2.03 ± 0.04). However, these values were not considered statistically different ($p > 0.05$) from the Sham ad libitum group (2.01 ± 0.01). The Ovx alcohol group presented lower values for Ca/P ratios (1.92 ± 0.06), being the only group statistically different ($p < 0.001$) from the Sham ad libitum group. Potential confounding variables are discussed.

Conclusion: Ovariectomy associated with alcohol consumption at 20% significantly changed the stoichiometry composition of hydroxyapatite in the alveolar bone crest, leading to a reduction in Ca/P ratios.

☆ Source of support: Adriana M.P.S. Marchini received a scholarship from the Brazilian governmental research agency, CAPES (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*).

* *Corresponding author at*: Av. Adhemar de Barros, 1136/153, São José dos Campos, SP 12245-010, Brazil. Fax: +55 12 39221555.
E-mail address: adrianampsmarchini@gmail.com (Adriana M.P.S. Marchini).

0003–9969/$ – see front matter
http://dx.doi.org/10.1016/j.archoralbio.2012.04.011

1. Introduction

Bones are composed of mineralized tissue constituting mainly of calcium (Ca) and phosphorous (P). These elements are organized forming crystals of hydroxyapatite.[1] Some conditions or pathologies affecting this tissue may alter the quantitative distribution of these elements, and consequently the stoichiometric composition of hydroxyapatite.[2–4]

Osteoporosis is a metabolic bone disorder, the most frequent etiologic factor of which is oestrogen deficiency, which occurs mainly in women after menopause.[5] This condition causes changes in the pattern of bone remodelling, with a predominance of the resorption process, which can alter the homeostasis of Ca and P and decrease bone mineral density.[4,6,7] Despite the importance of oestrogen deficiency in the aetiology of osteoporosis, it is a multi-factorial disease, involving several other risk factors, including excessive consumption of alcohol.[8]

The effects of abusive alcohol consumption on bone quality seem to be more dramatic in young individuals.[9] However, a decrease in bone mineral density when alcohol is consumed in large quantities, has also been reported in women after menopause.[10]

Periodontal disease is an infectious immune inflammatory alteration that affects the structures which support teeth. The primary etiological factor of which is bacterial biofilm.[11] However, its progression may be influenced by a wide range of variables which include systemic diseases (e.g. diabetes and osteoporosis), genetic disorders, habits (e.g. smoking and/or alcoholism), age, gender, stress, nutritional problems, including other factors, which may influence the way the host responds to an aggressive agent.[12–18]

Literature reviews have suggested that osteoporosis associated with both oestrogen deficiency and excessive alcohol consumption can be considered potential risk factors for the development of periodontal disease, which, if not controlled, could lead to tooth loss. However, the information available in the literature is insufficient for a definitive consensus, which highlights the need for further research by undertaking a greater number of well controlled and longitudinal studies.[15,16,19,20] It is possible that systemic bone loss associated with osteoporosis/osteopenia can also affect alveolar bone and its porosity which would lead to a greater susceptibility of bone resorption in the region.[15]

Despite the importance of Ca and P as major constituents of bone mineral phase and the possible implications of oestrogen deficiency and excessive alcohol consumption on the development of periodontal disease, to the best of our knowledge there are no studies that have evaluated concentrations of these chemical elements under these conditions, specifically in the region of alveolar bone crest, a structure whose integrity is important for the maintenance of periodontal health.

Considering the absence of such studies, this paper aims to evaluate the effect of oestrogen deficiency and excessive alcohol consumption on alveolar bone crest. Considering previous findings regarding oestrogen deficiency and alcohol consumption,[21–23] the hypothesis of this study is that oestrogen deficiency associated with alcohol consumption can adversely influence the quality of alveolar bone and alter its mineral composition.

2. Materials and methods

2.1. Treatment of animals

The present study was approved by the ethics committee of São José dos Campos School of Dentistry, State University of São Paulo – UNESP (Protocol No. 021/2008-PA/CEP).

Fifty-four rats (*Rattus norvegicus*, of the albinus, Wistar variety), aged four-months, were initially divided into two groups: ovariectomized (rats subjected to oestrogen deficiency by removing the ovaries), and Sham operated (simulated ovariectomy, ovaries exposed but not removed). A month after surgery, the two groups were sub-divided, and received the following dietary intervention for eight weeks: (a) alcoholic diet: solid diet and a 20% alcohol solution ad libitum, (b) isocaloric diet: solid and liquid diets with the same amount of calories consumed by the alcohol group and (c) ad libitum diet: solid diet and water ad libitum. The animals were randomized by weight in their respective groups.

The 20% alcohol solution was obtained by an absolute alcohol dilution in water. The concentration of the isocaloric solution contained, in millilitres, the same amount of calories as the 20% alcohol solution. It was prepared by dissolving 266 g sucrose in 1 l of water. Calculations were made taking into account the alcohol concentrations (20%), the density of absolute alcohol (0.787 g/ml) and the caloric values of sucrose (4.1 kcal/g) and alcohol (7.1 kcal/g). The solid diet was a commercial food (Labina – Purina®, Paulínia, Brazil).

The amount of calories (solid diet and alcohol solution) ingested by animals in the alcohol groups was measured daily. The following day, a diet with the same amount of calories (solid diet and isocaloric solution) was offered to isocaloric groups. Doing so, the treatment of animals with the isocaloric diet began and finished a day after the groups with the alcoholic diet.

To prevent dehydration, animals from the isocaloric groups also received water ad libitum. These animals received two bottles, one containing the sucrose solution and the other, solely water. However, in the statistical analysis of fluid consumption, for the isocaloric groups, only the amount of ingested sucrose solution was considered. This was done, as our intention was to compare the amount of calories ingested by the different experimental groups.

In summary, during the dietary treatment, the rats were divided into six experimental groups (each one presenting $n = 9$): Sham operated and ad libitum diet (Sham/ad libitum); ovariectomized and ad libitum diet (Ovx/ad libitum); Sham operated and alcoholic diet (Sham/alc); ovariectomized and alcoholic diet (Ovx/alc); Sham operated and isocaloric diet (Sham/iso); and ovariectomized and isocaloric diet (Ovx/iso). The Sham/iso group was pair-fed to Sham/alc group, while the Ovx/iso group was pair-fed to the Ovx/alc group.

Eight weeks after commencing the dietetic treatment, the rats were sacrificed and their mandibles removed. The ovariectomy success was confirmed, after sacrifice, by the visualization of ovary absence and uterus atrophy.

The rats were weighed at the beginning and at the end of the experiment. Weight changes were observed in percentage according to the formula below: