ARCHIVES OF ORAL BIOLOGY 59 (2014) 424–433

Available online at www.sciencedirect.com

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

Alcoholic and isocaloric diet, but not ovariectomy, influence the apoptosis of bone cells within the alveolar bone crest of rats

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ARTICLE INFO

Article history:
Accepted 23 January 2014

Keywords:
Alcoholism
Ovariectomy
Osteoporosis
Periodontium
Apoptosis

ABSTRACT

Objective: Studies suggest that chronic alcoholism as well as oestrogen deficiencies may affect bones in general, including alveolar bone and, by doing so, increase individuals' susceptibility to develop progressive periodontal disease. This paper aims to verify the influence of chronic alcoholism and/or oestrogen deficiencies in the apoptosis of bone cells of the alveolar bone crest region in rats.

Design: Initially, 54 rats were divided into ovariectomized (Ovx) and Sham operated (Sham) groups. Thirty days after surgery, these two groups were equally sub-divided, and received, for 56 days, the following dietary intervention: alcoholic diet (with 20% alcohol solution,), isocaloric diet and ad libitum diet (free diet). Analysis was undertaken by immunohistochemistry, using an antibody to detect apoptosis (anti PARP p-85).

Results: When comparing the six experimental groups, no significant differences were observed in the apoptosis of bone cells. Also, there was no significant difference in the quantity of cells undergoing apoptosis when the animals from Ovx groups were compared with those from Sham groups. However, when comparing only different dietary groups, differences were observed between the groups ad libitum and isocaloric, to osteoblasts ($p = 0.045$); and ad libitum and alcohol, to osteocytes ($p = 0.007$).

Conclusion: It is concluded that ovariectomy was not able to influence the rate of apoptosis of bone cells of the alveolar bone crest region in rats and that a possible influence of diet on apoptosis of osteoblasts and osteocytes cannot be ruled out.

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0003–9969/$ – see front matter
http://dx.doi.org/10.1016/j.archoralbio.2014.01.008

1. Introduction

Osteoporosis is a metabolic disorder that affects bone tissue. The most frequent etiologic factor of osteoporosis is oestrogen deficiency, which occurs mainly in women after menopause.[1,2] Despite the main role of oestrogen deficiency in the aetiology of osteoporosis, it is however a multifactorial disease which also involves others risk factors, including alcoholism.[3]

Periodontal disease is an inflammatory immune response which affects the structures that support the teeth, whose primary etiological factor is dental plaque. However, periodontal disease can be modulated by systemic factors, which alter the manner in which the host reacts to the offending agent.[4–6] The effects of oestrogen deficiency and excessive alcohol consumption on bone quality are more clearly understood and studied in long bones, however, some researchers suggest that such conditions also may affect the alveolar bone and increase the susceptibility of the host to the progression of periodontal disease.[7–10]

Alterations in bone quality when ovariectomy was associated with alcohol consumption have been previously observed by other authors in vertebrae,[11] in femur,[12] and in alveolar bone.[13]

Apoptosis is as important as mitosis to the growth and maintenance of the skeleton. It is suggested that all osteoclasts (cells responsible for bone resorption) are supposed to suffer apoptosis after completing a cycle of reabsorption. The majority of osteoblasts (cells responsible for bone formation process) also die, while others can become lining cells or osteocytes. Although osteocytes are considered to be long-living cells, they may also die prematurely. There is evidence that systemic hormones, mechanical forces, cytokines, growth factors and pharmacological agents may play a role in the regulation of bone cells apoptosis.[14] PARP (poly ADP ribose polymerase) is a nuclear protein involved in DNA repair processes, whose cleavage is a sensitive parameter for the detection of cells on apoptosis.[15–17]

Considering this, the objective of this study was to verify the influence of chronic alcoholism and/or oestrogen deficiencies in the apoptosis of bone cells of the alveolar bone crest region, in rats, by immunohistochemistry evaluation of the primary antibody anti PARP P-85.

Taking into consideration that the reduction of bone quality can occur due to an increase in reabsorption and decrease in bone formation, our hypothesis is that oestrogen deficiency associated with the consumption of alcohol can interfere in the process of apoptosis. This possibly happens due to a decrease in apoptosis of osteoclasts[18] and an increase in osteoblast apoptosis[19] and osteocytes, as it has been demonstrated that apoptosis of osteocytes may be a sign for the occurrence of the reabsorption.[20–22]

2. Materials and methods

2.1. *Animal treatment*

This study was carried out in accordance with the ethical principles adopted in Animal Experimentation and was approved by the ethics committee of São José dos Campos School of Dentistry, State University of São Paulo. As described earlier,[12,13] 54 female rats (*Rattus norvegicus*, of the albinus Wistar variety), aged three-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). Thirty days after surgery, these two groups were sub-divided, and received the following dietary intervention for 56 days: (a) alcoholic diet: solid food and 20% alcohol solution, (b) isocaloric diet: solid and liquid diets with the same amount of calories consumed by the alcohol group, (c) ad libitum diet (free diet): solid food and water ad libitum.

In alcohol groups, the 20% alcohol solution (obtained by an absolute alcohol dilution in water) and solid food were offered in enough quantity so that the animals could eat and drink until satiety. 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 of sucrose in 1 litre of water. The solid food was a commercial food (Labina – Purina®, Paulínia, Brazil).

The animals from isocaloric groups were forced to ingest a restricted diet to simulate the same caloric intake exhibited by the alcohol groups. To do so, the amount of calories (from solid food and alcohol solution) ingested by animals in the alcohol groups was measured, and the following day, a diet containing the same amount of calories (solid food and isocaloric solution) was offered to the isocaloric groups. In doing so, the treatment of animals with the isocaloric diet began and finished a day after the groups receiving the alcoholic diet.

To prevent dehydration, animals from the isocaloric groups also received water ad libitum. These animals received two bottles, one containing the isocaloric solution and the other, solely drinking water (calorie-free). However, in the statistical analysis of liquid consumption, for the isocaloric groups, only the amount of ingested isocaloric solution was considered. This was done as the intention was to compare the amount of calories ingested.

During the dietary treatment, the rats were divided into six experimental groups ($n = 9$): Sham and ad libitum diet (Sham/ad); ovariectomized and ad libitum diet (Ovx/ad); Sham and alcoholic diet (Sham/alc); ovariectomized and alcoholic diet (Ovx/alc); Sham and isocaloric diet (Sham/iso); and ovariectomized and isocaloric diet (Ovx/iso).

Fifty-six days after commencing the dietary treatment, the rats were anaesthetized and sacrificed, and their mandibles were removed. Ovariectomy success was confirmed, after sacrifice, by the visualization of ovary absence and uterus atrophy.

The rats were weighed at the beginning (the day they were ovariectomized or Sham operated) and at the end of animal treatment phase (sacrifice). Weight changes were observed in percentage according to the formula below:

$$\frac{[(\text{Final weight} - \text{Initial weight}) \times 100]}{\text{Initial weight}}$$

The average value of solid and liquid diet consumed per rat/per day was recorded.