Chronic stress increases bone resorption in apical periodontitis stress and endodontic disease in rats

Tereza Aparecida Delle Vedove Semenoff1†, Aurélio Rosa da Silva-Junior2†, Fábio Luís Miranda Pedro1†, Álvaro Henrique Borges1†, Alessandra Nogueira Porto1† and Alex Semenoff-Segundo1†

*Correspondence: t.semenoff@uol.com.br
†These authors contributed equally to this work.
1Master’s Program in Integrated Dentistry Science – University of Cuiabá – UNIC. Cuiabá-MT, Brazil.
2Master’s Program in Health Sciences – Area of Concentration: Surgery, Nutrition and Metabolism at the Federal University of Mato Grosso, Cuiabá-MT, Brazil.

Abstract

Background: The aim of this study was to radiographically evaluate the effects of chronic stress on induced endodontic disease in rats.

Methods: Twenty-four Wistar rats were divided into two groups: stress associated with coronal access (SAG) and coronal access (AG). Initially, the SAG and AG groups underwent coronal opening on their first right upper molars. Beginning the next day, the SAG group was subjected to physical restraint for 12 daytime hours daily for 29 days, which induced stress. After this period, the animals were euthanised, and their jaws were radiographed according to a standardised method. The radiographs were digitised, and the area of the periapical lesions was measured.

Results: The results indicate that the SAG group experienced a greater destruction of the apical periodontium, which was significantly different from that observed in the AG group (p<0.05).

Conclusions: Chronic stress was able to cause an increase in disease of the apical periodontium.

Keywords: Disease, endodontics, radiography, dental, rats, stress, periapical, lesion

Introduction

It is estimated that a large portion of the population suffers from stress. It seems that even small problems day to day are capable of producing severe disease states if they are not well managed [1].

This emotional state is capable of providing an interaction between the central nervous system (CNS), the endocrine system, and the immune system. It is known that these systems participate in the establishment of health or disease in humans [2]; considering this perspective, expressed emotions alter the pattern of inflammatory responses [3].

Endodontic disease is caused by a biofilm of microorganisms that are organised within the root canal system [4]. In addition to contributing to the loss of the tooth, this source of infection may harbour microorganisms that can cause systemic complications. The resulting disease has a direct impact on quality of life and implies a great loss of resources for society [5].

The relationship between stress and the progression of periapical lesions is still not well documented, although the ability of stress to suppress immune periodontal defence responses, causing the progression of infection, is well publicised in the literature [6].

In the presence of apical biofilm, an inflammatory process occurs that is capable of producing products such as interleukins (IL-1, IL-2, IL-6 and TNF) that are important contributors to the destruction of apical periodontal tissue [4].

Thus, it is known that the mechanisms of stress affect tissues through these products and by-products. However, these factors act in the presence of cortisone in periodontal tissues, and this hormone is capable of reducing the immune-inflammatory response stimulated by chronic stress [7]. It is acknowledged that in immunosuppressive diseases there is a further progression of infectious diseases [3]. Therefore, this study may supplement the current knowledge about the relationship between chronic stress and endodontic disease.

Little is known about the association between the pathogenesis of endodontic disease and chronic stress. This study used a previously tested stress model to further the understanding of the relationship between the effect of stress and the progression of endodontic disease [6]. Thus, the objective of this paper was to observe the effect of chronic stress on induced periapical lesions in rats.

Methods

This study was approved by the research ethics committee at UNIC (CEP/UNIC) under Protocol #0307-320. For this experiment, 24 male Wistar rats were selected from the central vivarium of the University of Cuiabá, MT, Brazil (Universidade de Cuiabá -
The rats were maintained with balanced food rations and *ad libitum* water on a 12-hour light/dark cycle, and with a controlled temperature of 23ºC and a humidity of ±50%. They underwent a one-week adaptation to the new environment.

**Study design**

Animals between 8 and 10 weeks old (average weight 250g) were distributed into two groups by an external lab assistant: the Stress associated with Coronal Access Group (SAG, n=12) and the Coronal Access Group (AG, n=12).

On the first day of the experiment, the SAG and AG animals underwent the coronal opening procedure on their first upper right molars. On the second day, the SAG animals began the stress protocol, and this condition was maintained for 29 days.

**Experimental periapical lesion**

All of the induction procedures for endodontic disease were performed through a coronal opening to the 1st upper right molar [8]. The procedures were performed using anaesthesia that consisted of an intramuscular administration of 0.1ml of ketamine chloride (Dopalen, Agribraza. Animal Health, Paulínia, SP, Brazil) with 0.05ml of xylazine-chloral hydrate (Rompun, Bayer. Animal Health, São Paulo, SP, Brazil) for each 100grams of body weight.

A high-speed handpiece with a spherical bit (1010-KG - Sorensen, Cotia, SP, Brazil) was used to create an opening in the pulpal chamber that passed through the enamel layer and the dentine to reach the dental pulp. The teeth remained open until the end of the experiment.

**Stress protocol**

To perform the stress protocol, the rats were enclosed in PVC pipes made to size that were closed with wire on each end for a 12-hour interval each day from 6 AM to 6 PM. This procedure was repeated for 29 days [7,9].

On the 30th day of the study, the animals were euthanised by an anaesthesia overdose. The jaws were removed and immediately stored in a 10% buffered formaldehyde solution.

The following week, the material was prepared by removing excess tissue so that periapical radiographs could be taken.

The specimens were placed on top of the radiographic film and stabilised with utility wax. The maxillae were positioned with the teeth parallel to the cone of the X-ray apparatus, attempting to create a 90º angle in relation to the X-ray-producing source (Spectro 70x, Dabi Atlante, Ribeirão Preto, São Paulo) (Figures 1 and 2).

**Measuring the periapical lesions**

Before starting the experiment, the examiner conducted training and studied the anatomical structures involved in the evaluations. After the onset of the study, a collaborator assembled the material so that the examiner was unaware of the groups involved in the study.

After being processed according to a set standard, the radiographs (Kodak INSIGHT Dental Film, São José dos Campos – SP, Brazil) were digitised on an HP Photosmart Express C3180 Scanner and were then transferred to imaging software (ImageLab 2000). This software calculated the area of the lesions, which were grouped together and then subjected to statistical analysis using SPSS version 18.0 (Statistical Product and Service Solutions, Chicago, Illinois, United States of America).

For data comparison, Student’s *t*-test for independent samples was used. Significance was assigned at a level of 5%. * Represents significant difference between the groups (p<0.05).

**Results**

One animal from the SAG group died during anaesthesia for the coronal opening procedure.

The weight of the animals (Table 1) was calculated by subtracting the initial weight from the final weight. Thereby, the SAG gained nearly 20g (21.22±6.30) and the AG group

|    | N  | Mean | Standard deviation | Standard error of the mean |
|----|----|------|--------------------|---------------------------|
| SAG| 11 | 21.22*| 6.30               | 2.10                      |
| AG | 12 | 56.22*| 7.44               | 2.48                      |

* Student's *t*-test for independent samples was used. Significance was assigned at a level of 5%. * Represents significant difference between the groups (p<0.05).
The results of this study demonstrate that chronic stress in rats gained nearly 60g (56.22±7.44). In comparing the difference between the weights, there was a statistically significant difference between the groups (p<0.05).

The study results (Table 2) demonstrated that larger periapical lesions (17.67±5.93) existed in the animal group that underwent chronic stress associated with the coronal access (Figure 1) (SAG) compared with the group that only underwent the coronal access procedure (12.93±4.91 p<0.05).

The radiographs of the sides opposite the access points revealed no disease on the apices of the teeth (Figure 2).

Discussion
The results of this study demonstrate that chronic stress in rats contributed to the amount of growth of periapical lesions on radiographs. Studies have demonstrated that chronic stress seems to affect mammals’ defence systems [10].

Although the pathogenesis of periapical lesions is mostly microbial in nature [8], the influence of systemic factors such as stress should be better elucidated.

Concerning the origin of periapical lesions, the risk factors seem to be connected to the quality of coronal sealing and the endodontic treatment performed [11-12]. Furthermore, a high prevalence of endodontic disease recurrence is evident in many parts of the world [13-15].

In contrast to the structures surrounding and supporting the teeth, in which stress is a contributing risk factor in the development of periodontitis [16-17], there is little information in the literature regarding the progression of periapical lesions associated with chronic stress.

Stress promotes increases in the secretion of adrenocorticotropic hormone (ACTH) by the adenohypophysis, leading to a greater production of corticosterone by the adrenal glands of rats. This neurohormone acts in the balance of Th1 and Th2 cells, which represent the cell response and humoral response, respectively [18-19].

In the establishment of periapical lesions, there is a need for the regulation of cellular elements, such as PMN, macrophages, lymphocytes and enzymes, which secrete products and byproducts, such as inflammatory cytokines (IL-1, IL-6, IL-8, TNF, IFN, colony-stimulating factors, and growth factors, among others). It seems plausible that stress, through the secretion of hormones such as cortisol and adrenaline via the mechanisms outlined above, acts in the regulation of these substances, which bind to the developing periapical lesions. Thus, changes in the inflammatory process in the apical periodontium may be able to produce an increase in radiographic lesions [4,19-20].

The physical confinement stress model is well established in the literature and was chosen for its simplicity and low cost, which facilitate the performance of experimental tests of this nature [7,21-23]. The 12-h stress protocol was chosen because although there is still little information related to the subject, there is evidence that this model is able to contribute to alveolar bone loss in rats. It is important to emphasise that a daily 12-hour procedure is a model of intense stress when compared with those used in other studies in the medical field [24-25].

Notably, the SAG gained significantly less weight than the AG. Therefore, the systemic effect of stress is in agreement with that of other studies [7,9,21].

The model for the induction of endodontic disease is not new [8]. Other studies currently use this same type of coronal opening in experimental models in rats [26-27].

One of the limitations of this study was the lack of laboratory tests for the evaluation of systemic indicators, such as the weight of endocrine organs and a biochemical cortisone exam. However, in addition to results demonstrating the effect of stress, this model is widely validated, as has been previously described [7].

Conclusion
From the results obtained and within the limitations of this study, it may be concluded that chronic stress was able to cause an increase in disease of the apical periodontium.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
Tereza Aparecida Delle Vedove Semenoff - Conception, design, acquisition and interpretation of data; manuscript writing. Aurélio Rosa da Silva Junior - Acquisition and interpretation of data; manuscript writing, critical revision. Alex Semenoff-Segundo - Acquisition and interpretation of data; manuscript writing, supervised all phases. Alessandra Nogueira Porto - Interpretation of data, critical revision. Álvaro Henrique Borges - Manuscript writing, critical revision.

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Table 2. Radiolucent area of periapical lesions measured on digitized radiographs in mm².

|     | N     | Mean  | Standard Deviation |
|-----|-------|-------|--------------------|
| SAG | 11    | 17.67 | 5.93               |
| AG  | 12    | 12.93 | 4.91               |

Student’s t-test for independent samples was used. Significance was assigned at a level of 5%. *Represents significant difference between the groups (p<0.05).
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