

ORIGINAL RESEARCH

Effect of Chronic Stress on Ligature-induced Periodontitis in Inbred and Noninbred Rats: A Radiographic Study

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ABSTRACT

Aim: The aim was to compare the effect of chronic stress on ligature-induced periodontitis in inbred and noninbred female rats by means of a radiographic study.

Materials and methods: Adult Lewis (inbred) and Wistar (non-inbred) rats were used and divided into the following groups: Ligature/Stress Lewis Group (LSLG, n = 8); Ligature/Stress Wistar Group (LSWG, n = 8); Ligature Lewis Group (LLG, n = 8) and Ligature Wistar Group (LWG, n = 8). The animals were anesthetized and a silk thread was continuously kept around their second upper right molar. Then, groups LSLG and LSWG were subjected to physical stress test (physical restraint for 12 hours). After 15 days of ligature placement, the animals were euthanized. The right hemimaxilla was kept in formalin solution for 48 hours. Radiographs of the hemimaxillae were obtained using the parallelism technique and subsequently submitted to a standardized radiographic processing. The examiner was blinded to the groups and calibrated. The bone height level was measured and the data were subjected to statistical analysis (ANOVA, Tukey, p < 0.05).

Results: LSWG showed bone destruction significantly higher than that of LSLG (32.1 ± 1.45 mm and 23.6 mm ± 2.13, respectively). Similarly, LWG showed bone destruction significantly higher than that of LLG (28.6 ± 1.18 mm and 25.2 ± 2.76 mm, respectively).

Conclusion: Inbred rats (Lewis) are less susceptible to the effects of chronic stress than are noninbred rats (Wistar) in relation to ligature-induced periodontitis.

Clinical significance: Epidemiological studies have shown a relationship between stress and periodontitis. One of the major difficulties of this type of research is the bias of the exact diagnosis of the patient's emotional state. Inbred lines animals have standards-specific neuroendocrine, this allows answers about the susceptibility of periodontal disease, making knowledge applicable in future clinical trials.

Keywords: Stress, Physiological, Rats, Inbred lew, Rats, Wistar, Laboratory research.

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INTRODUCTION

Periodontal disease is the result of an infection leading to inflammation of the tissues surrounding the teeth, which can be exacerbated by the presence of specific microbial species.¹ Although it has been established that dental biofilm is the main etiological factor of periodontitis,² the host immune response contributes directly to its progression.³

A number of factors favor the development and progression of periodontal disease;² many of them are considered risk factors, which include cardiovascular disease,³ diabetes,⁴ and environmental factors such as smoking, stress and metabolic syndromes.⁵ The latter seem to modify the pathogenesis of infections that affect the periodontium, especially due to the growing number of obese people in the world.⁶ Genetic factors are also linked to further progression of periodontitis,⁷ but this relationship remains unclear. There is evidence that patterns of systemic inflammatory responses could increase susceptibility and progression rates of this disease.⁸

Given this set of causal information and cofactors, it becomes important to eliminate some biases that are present in human behavior. The animal model used, particularly rats, is interesting to understand the pathogenesis of the disease. In the last decade, several studies investigating induced periodontitis and chronic stress have been reported in the literature.^{9,10} These animals have specific patterns of neuroendocrine responses able to prevent further progression of infections, such as periodontitis, particularly the lineage selected in this study.¹¹

This study sought to understand the effect of chronic stress on an inbred lineage (Lewis) nonsusceptible to infec-

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tion and on other lineage with no specific patterns of immunoinflammatory responses (Wistar). It is known that chronic stress is able to modify the hypothalamic-pituitary-adrenal axis, promoting strong changes in susceptibility to diseases such as periodontitis.¹²

Thus, the aim of this study was to compare the effect of chronic stress on ligature-induced periodontitis in inbred (Lewis) and noninbred (Wistar) rats by analysis of radiographic bone loss.

MATERIALS AND METHODS

The study was approved by the Research Ethics Committee of the University General Hospital at the University of Cuiaba (protocol #2010-044). A total of 18 Wistar rats (*Rattus norvegicus*) and 18 Lewis rats (*Rattus norvegicus*) were selected for the study. These animals were acclimatized to housing conditions over 10 days, kept under a 12 hours light/dark cycle at 24°C, at +40% relative humidity, with access to standard rat chow pellets and water ad libitum. The animals were selected for the study weighted approximately 250 gm—average age of 2½ months.

All procedures in this experiment were performed under general anesthesia by means of intramuscular injection of a combination of 0.1 ml of ketamine hydrochloride and 0.05 ml of xylazine hydrochloride for each 100 gm of body weight. After anesthesia, sterile 4-0 silk ligatures were placed around the second upper right molar. The second upper left molar was used as control. One day after induction of periodontitis, rats in the stress group were placed individually in ventilated plastic restraint tubes for 12 hours for 1 day during daytime (6 to 18 hours). At the end of this period, the animals were sacrificed by anesthetic overdose after 15 days of receiving the ligatures.¹²

Right and left hemimaxillae were fixed in 10% formalin solution for 48 hours. Radiographs were taken using a dental X-ray unit (Spectro 70x, DabiAtlante, Ribeirão Preto, SP, Brazil). In order to take the images, specimens were placed on a film (Insight; Kodak Company, Rochester, NY, USA) and the X-ray cone was set perpendicular to it. The distances between the cone and the specimens as well as an exposure time of 0.3 seconds were standardized based on a previous pilot study. A trained operator processed all radiographs using the same time and temperature.

Radiographs were projected on a white board using a slide projector (Kodak-Ektagraphic III, Rochester, NY, USA) that provided a 15-fold magnification of the images.

Then, after identifying the structures between the first and second molars, the teeth and interproximal bone crest were drawn on the board, as well as a horizontal line between

the cusp tips of both teeth. At the contact point between the molars, a vertical line perpendicular to the horizontal line was drawn.

The distance between the bone crest and the horizontal line was determined by means of a digital caliper (Mitutoyo, Sul Americana Ltda, Santo Amaro, SP, Brazil) and recorded for further analysis and comparisons (Fig. 1). A previously calibrated and blinded operator performed all measurements,¹² as seen in Table 1.

The sample size calculation was performed based on studies of ligature-induced periodontitis and chronic stress, which in general, estimate a difference of 0.3 mm with standard deviation of 0.2 mm. It was opted for a 0.8 power (80%) with a significance level of 5%. The bone loss scores were grouped in such a way that the measurements obtained were statistically analyzed using ANOVA/Tukey at a 5% significance level on SPSS software.

RESULTS

Comparisons of the different species (Table 2) showed that LSWG had bone destruction significantly higher ($p < 0.05$) than LSLG; also, LWG had bone destruction significantly higher ($p < 0.05$) than LLG (28.6 ± 1.18 mm and 23.6 ± 2.13 mm, respectively).

For comparisons of the same species (Table 2), it was verified that LSWG had bone loss significantly higher ($p < 0.05$) than LWG. Similarly, LSLG showed bone loss significantly higher ($p < 0.05$) than LLG.

DISCUSSION

According to the study's hypothesis, the results demonstrated that stress can modulate further destruction caused by periodontitis in noninbred Wistar rats when compared to inbred Lewis rats.

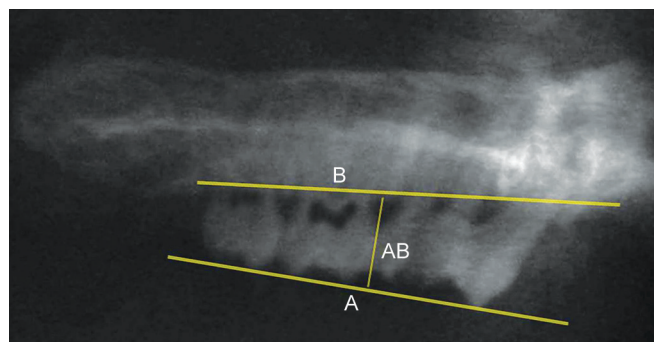


Fig. 1: Illustration of the measurement method of radiographs evaluated in the groups LSLG, LSWG, LLG and LWG. A—horizontal line between the cusp tips of upper molars; B—horizontal line through the most coronal portion of the bone crest between first and second molars; AB—a vertical line perpendicular to the horizontal lines referring to the distance between intercusps path and bone crest

Table 1: Radiographic distances in millimeters between the occlusal plane and the distal root apex of the second molar

Measurement	Number of specimens	Radiographic distance (Mean ± Standard deviation)
First	8	43.34 ± 0.08 ^a
Second	8	43.46 ± 0.09 ^b

Different letters represent statistically significant difference ($p < 0.05$)

It is known that inbred Lewis rats have defined patterns of neuroendocrine responses in the periodontium.¹¹ The hypothesis was to know if stress could produce changes in bone destruction patterns of different lineages when subjected to induced periodontitis, which was demonstrated herein.

It seems to be established that in rats, physical restraint stress for 12 hours contributes to further progression of induced periodontitis.^{9,13,14}

An interesting variable included in this study was the presence of inbred lineage of rats (Lewis). It is known that these animals have patterns of disease progression little susceptible to infections such as tuberculosis and leishmaniasis,¹⁵ but it was wondered about what would be the animal behavior in relation to bone loss as a result of stress on the periodontium.

The results demonstrated that LSLG showed higher bone loss values compared to LLG, but lower than those for LSWG. There have been several studies reported in the literature corroborating these results.^{13,16-18}

Stress causes a depression of various defense systems due to alteration of the central nervous system. The organism response is variable and depends on the type of stress imposed. In the human body, this response affects neuroendocrine and behavioral systems, both being modulated by the hypothalamic-pituitary-adrenal axis,¹⁹ which undergoes changes at stress, increasing hyperresponsivity with greater tissue destruction, as shown in this study,²⁰ besides contributing to schizoid behaviors in many cases.²¹

Stress is one of the factors associated to increased susceptibility of developing periodontal disease, even though this has not been fully established yet.^{5,6,16} It affects the infectious/inflammatory processes,¹¹ and the hypothalamic-pituitary-adrenal axis (HPA axis) modulates neuroendocrine responses, which seem to have a relationship with the worsening or improvement of periodontal disease.

This happens because the HPA axis operates through neurohormones and cytokines that activate and interfere with cortisol level, avoiding defenses against microbial antigens of periodontitis.¹³

It seems to be clear that the neuroendocrine immune axis plays a role when manipulated and stimulated to pro-

Table 2: Mean bone destruction in the study groups expressed in millimeters (20× amplified), according to the groups (n = 8)

LSWG*	LSLG [†]	p
32.1 ± 1.45 Aa	25.2 ± 2.76 Ba	<0.05
LWG [‡]	LLG [§]	
28.6 ± 1.18 Ab	21.6 ± 2.13 Bb	<0.05

*LSWG: Ligature Stress Wistar Group; [†]LSLG: Ligature Stress Lewis Group; [‡]LWG: Ligature Wistar Group; [§]LLG: Ligature Lewis Group. Student's t test for independent samples ($p < 0.05$); Different capital letters in lines indicate statistically significant difference ($p < 0.05$); Different lowercase letters in columns indicate statistically significant difference ($p < 0.05$)

duce higher or lower progression of bone loss upon chemical or emotional modulation.^{11,18,22} Unfortunately, many aspects related to etiopathogenesis of periodontitis are still unknown.²³

Currently, it is known that part of the population is little susceptible to develop periodontitis.²⁴ Nevertheless, it seems that once a neuroendocrine response is activated, biological phenomena come up related to environmental risk factors contributing to further progression of periodontitis, many of which are demonstrated by current epidemiology²⁵ and others are still unclear.

In this respect, studies with rats can provide knowledge of biology without biases coming from environmental factors and also contribute to the understanding of this biology. Hence, it is perceived that in animals, little susceptible to infection, as those used in this study, chronic stress contributed to further progression of bone loss in the periodontium.

An explanation of the findings of this study may refer to the major histocompatibility complex. This is a determinant present on specific defense cells leading to susceptibility and progression of infections such as leishmaniasis, tuberculosis¹⁵ and periodontitis.¹¹ Therefore, both the lineages studied were found to have specific patterns in the immune/inflammatory response.

Inbred Lewis rats and noninbred Wistar species has different HPA axis activation.^{26,27} The response pattern of Lewis rats have low reactivity of HPA axis making the animals more susceptible to autoimmune diseases,¹⁶ but less susceptible to infections such as periodontitis.¹¹ This low reactivity is believed to provide a predominance of specific responses to T-helper lymphocyte 1 cells, which is associated to decreased progression of periodontitis.¹¹

There are evidences suggesting that it happens in the periodontium,²⁸ but trials in humans seem to demonstrate responses differing from those found in animals.²⁹ Although these factors are related to a lower progression of induced periodontitis in Lewis rats, chronic stress in this study was able to act as a contributing factor associated with increased

progression of bone loss. Wistar rats seem to respond similarly to other findings in the literature.^{9,12-14}

It seems that modern life exposes individuals to high impact emotional challenges. Thus, studies investigating chronic stress and periodontal diseases may contribute to the plausibility of information about the topic discussed herein.

CONCLUSION

Inbred Lewis rats showed lower bone loss values when subjected to stress. In both lineages, stress contributed to increased bone destruction.

CLINICAL SIGNIFICANCE

Epidemiological studies have shown a relationship between stress and periodontal disease. The main difficulty in this type of research is to obtain an accurate diagnosis of the patient's emotional state. Inbred rats (Lewis) have a neuroimmune endocrine-specific pattern, which allows more plausible answers on the susceptibility of periodontal disease, making knowledge obtained applicable in future clinical trials.

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