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Evaluation of the Influence of Experimental Periodontitis on the Sexual Behaviour of Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author SP did the experimental studies, data and statistical analysis, manuscript preparation, editing and review. Authors SCS, KFC and CCLB did the experimental studies, data analysis and manuscript preparation. Authors PON and CAN were definition of intellectual content, design, experimental studies, data and statistical analysis, manuscript preparation, editing and review. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: Few studies have been reported a correlation between periodontal disease and erectile dysfunction in men. The aim of the present study was to evaluate the sexual behaviour of adult male rats with experimental periodontitis.
Materials and Methods: Fourteen male rats were divided into the following two groups: non-ligature, control (n = 7) and experimental with ligature (n = 7). The latter group had a ligature placed around each lower first molar for the development of experimental periodontitis. After 29 days of induction of periodontitis, the sexual behaviour of the male rats was recorded between 20:00-21:00, with lighting provided by a dim light. Initially, the males were placed in an observation

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cage for an adaption period of 900 seconds. After this time, a sexually receptive female was introduced into the cage with the male. On the thirtieth day the rats were sacrificed and samples of hemimandible were collected for radiographic analysis.
Results: The rats in the ligature group showed a decrease ($p < 0.05$) in the intrusion frequency compared with the control group and ejaculation was not observed in the ligature group during the maximum recording time. It was confirmed that periodontitis was induced in the group with ligature due to the greater distance from the cementoenamel junction to the alveolar crest in the radiographic analysis ($p < 0.01$).
Conclusion: Experimental periodontitis may contribute to a reduction in the sexual behaviour of male rats.

Keywords: Periodontitis; erectile dysfunction; sexual behaviour; rats; ligature.

1. INTRODUCTION

Periodontal disease is characterized by an inflammatory response in periodontal tissues. It is often asymptomatic and is caused by microorganisms that are present in bacterial plaque. The clinical manifestation of the disease depends on the nature or stimulation of this response [1]. Gingivitis is the mildest form of periodontal disease; it does not affect the underlying support structures of the teeth and is reversible. Periodontitis results in the loss of connective tissue and bone support through the action of toxic products (bacterian toxins) that are released in the subgingival area for specific periodontal pathogens, as well due to an inflammatory response that is triggered by the presence of these pathogens [2,3].

Few studies have reported that chronic periodontitis may be strongly associated with systemic vascular diseases of the coronary, cerebrovascular and chronic obstructive pulmonary disease through the induction of endothelial dysfunction [4-7]. Periodontal infection may indirectly induce endothelial dysfunction via a state of systemic inflammation, as evidenced by a high concentration of pro-inflammatory cytokines in the plasma [5-8].

Thus, it is reasonable to hypothesize that systemic inflammation induced by periodontal pathogens may be associated with endothelial dysfunction and atherosclerosis, firstly in small vessels, such as micro-vasculature of the penile vessels, and later progressively affecting the larger arteries like the coronaries [9].

Therefore, we suggest that there is a correlation between periodontal disease and erectile dysfunction, which has been supported by Peng and Zhng [10], who have suggested that periodontal disease may induce erectile dysfunction because it contributes to endothelial dysfunction.

On the basis of the aforementioned finding, we wanted evaluate the association between induced periodontal disease and male rat sexual behaviour.

2. MATERIALS AND METHODS

2.1 Animals

Pregnant rats were obtained from the Central Animal Facility of the State University of West Paraná (UNIOESTE), Cascavel campus, and kept in the vivarium of the Laboratory of Endocrine Physiology and Metabolism under controlled conditions of temperature (23±2℃) and light (12-hour cycle of light and 12 hours of darkness - 07:00 to 19:00). At birth, the pups were separated by sex, with only 7 males for each litter, making a total of 14 animals, which were divided into 2 groups and all the animals received standard diet and water at will throughout the experimental period. All the experimental protocols were approved by the Ethics Committee on Animal Experiments and Practical Classes (CEEAAP) of UNIOESTE. The calculation of sample size was performed using the T test based on previous study researchers group [11].

2.2 Induction of Periodontal Disease

At 70 days of age, the animals were anesthetized (xylazine 0.04 mL/100 g and ketamine 0.08 mL/100 g), and placed on an appropriate operating table, which permitted the opening of the e.g. oral cavity, facilitating access to the posterior mandible teeth. With the aid of modified forceps and an exploratory probe, a number 40 cotton thread was tied around the first right and left lower molars. Two groups were

then designated: the first being control without ligature (CON) and the second being experimental with ligature (LIG). This ligature acted as a gingival irritant for 30 days, which favored the accumulation of bacterial plaque and the subsequent development of periodontal disease [11].

2.3 Analysis of Sexual Behaviour

Twenty nine days after the induction of periodontal disease, the sexual behaviour of the rats in both the control and the experimental groups was recorded between 20:00 and 21:00. The observations were performed during the period of darkness with illumination provided by a weak light because sexual behaviour is slightly more intense during the dim light and they were made by a blinded examiner. Initially, the male rat was placed in the observation cage (70 x 70 x 35 cm) for an adjustment period of 900 seconds. After this period a sexually receptive female was introduced into the cage with the male and the following parameters of the behaviour of the male rat were recorded, it based on the study by Agmo [12].

a) Frequency of smelling the anogenital region;
b) frequency of mounts with pelvic movements. According to Agmo [12], the male normally mounts from behind, putting its front paws on the back of the female and making rapid anteroposterior pelvic thrusts (17-22 Hz) for about 300 ms. During mounting, the female assumes a lordosis posture. The male then dismounts very slowly;
c) latency (seconds) of intrusion behaviour. The time from the introduction of the female into the observation cage until the first intrusion by the male (deep forward momentum, featuring vaginal penetration). If the rat did not intrude into the female within 600 seconds it was considered to be sexually inactive;
d) latency (seconds) of ejaculation. The time from the first intromission to ejaculation. Ejaculation was evidenced by penetration with deep pelvic movements and then lying slowly to the side, followed by a refractory period [1]. If a rat has introduced, but did not ejaculate soon afterwards, the rat was considered to be sexually inactive, with the recording continued for up to 30 minutes;
e) duration (seconds) of general movement. The total duration of movement during the observation period was recorded in order to evaluate possible motor abnormalities caused by the protocol of the induction of periodontal disease.

The animals were filmed until the moment of ejaculation. The latency of intrusion and the frequency of intrusion were analyzed within the first 600 seconds of the 1800 seconds recorded period because, in the majority of the control rats, the recording session corresponded to 10 minutes. When the frequency of intrusion was zero, the latency of intrusion was considered to be 600 seconds. In rats that did not exhibit intrusion, the ejaculation latency was considered to be 1800 seconds.

2.4 Radiographic Analysis

Thirty days after the induction of periodontal disease, the rats in the control and experimental groups were fasted for eight hours with only unlimited water. They were then desensitized with carbon dioxide and when it was observed that they were sedated they were decapitated. The left hemimandible was completely dissected and fixed in 10% formalin solution for subsequent radiographic examination. The hemimandible from the left side of each animal was fixed in buffered formaldehyde (pH 7.2) for 48 hours and was then subjected to radiographic analysis. This entailed placing the hemimandible with the lingual side on periapical X-ray film (AGFA DENTUS®, Ultraspeed) positioned so that the buccal and lingual cusps of the first molars stayed in the same vertical plane. A GE – 1000 X-ray machine was used, set to 15mA, 65Vp, 18 pulses, focus/film distance of 50 cm with perpendicular incidence of X-rays to the pieces. For the processing of the films Kodak® developer and fixer was used in the respective processing of time/temperature and the images were scanned through a scanner for slides (Polaroid Sprint Scan 35 Plus, Polaroid). The scanned images were analyzed in three measures using the Image Tools 3.0 program and an average was taken from them by linear measure, which covered the distance from the cementoenamel junction to the alveolar bone crest of the mesial side of the first lower left molar of the rat, with the measurements in pixels [11].

The comparison of means within each group was presented in table with units and corresponding

measures, with the mean ± standard deviations using the Student–Newman– Keuls test ($p < 0.05$) in the statistical analysis of the following parameters frequency of smelling the anogenital region, frequency of mountings with pelvic movements and duration of general movement; Mann-Whitney test ($p<0.05$) for the following parameters latency of introduction behaviour and latency of ejaculation because they didn't have normal distribution and the radiographic analysis, after checking the normal distribution of the data through the Bioestat 5.3 program (Mamiraua Institute, Amazonas, Brazil), Student's t test was performed with $p <0.05$ to evaluate the difference between the groups.

3. RESULTS

The frequency of sniffing the anogenital region averaged 8.2±1.0 unit/1800 seconds for the control group and 12.7±1.2 unit/1800 seconds for the experimental group. In the control group, it was observed that when the rats were introduced into the observation cage they smelled the female to recognize her before starting intercourse for a shorter time than the rats in the ligature group (Table 1).

Considering that the introduction of the penis was not directly visible, we recorded the behaviour leading up to penetration, which was characterized by the male rat placing its forepaws on the back of the female with associated rhythmic pelvic movements [13]. The rats in the ligature group showed a decrease ($p < 0.05$) in the frequency of intrusion (1.1±0.8) compared with the control group (12.3±1.9). Consequently, the latency of introduction behaviour was 600 seconds for the ligature group, i.e. in this group penile intrusion was not observed, which reflected a decrease in sexual desire, as in the control group the latency of intrusion was 19 seconds (Table 1).

The latency of ejaculation was 980 seconds for the control group and 1800 seconds for the ligature group. Because the experimental group did not provide evidence of intrusion there was no ejaculation in the ligature group during the maximum recording time (1800s; Table 1). The duration of the general movement averaged 74.8±8.4 for the control group and 112.0±7.1 for the ligature group. Thus, it is apparent that the animals in the ligature group moved more in their cage without performing the sexual act, which suggests reduced sexual behaviour or libido in the ligature group, considering that the male rats were in the presence of sexually receptive females and did not mount them. The fact that some rats moved more is because they were getting to know the environment by smelling, like any other rat, however, because they had no interest in the female rats they spent that time familiarizing themselves with the environment (Table 1). For the statistical analysis, all the numerical values were expressed as mean± standard error of the mean, except for the latency data (ejaculation and introduction behaviour), where the median (interquartile range) was used. Student's t-test was performed, followed by the Newman-Keuls test or the Mann-Whitney test (latency).

In the Table 2 shows that the radiographic analysis of the first lower molar showed that the ligature group had significantly higher alveolar bone loss than the control group ($p <0.01$), indicating the action of induced periodontitis on the alveolar bone tissue.

Table 1. Effect of the induction of periodontal disease in the frequency of the behaviours of sniffing the anogenital region; mounting movements; the latencies (s) of penile insertion in the presence of a sexually receptive female (R); ejaculation and the overall duration of general movement

Behaviour	Control (n=7)	Control+Ligature (n=7)
Frequency of smelling the anogenital region[d, f]	8.2±1.0	12.7±1.2[a]
Frequency of mountings with pelvic movements[d, f]	12.3±1.9	1.1±0.8[a]
Latency of introduction behaviour[d, e]	19 (16/32)	600 (600/600)[b]
Latency of ejaculation[c, e]	980(825/1080)	1800 (1800/1800)[b]
Duration of general movement[d, f]	74.8±8.4	112.0±7.1[a]

[a]p < 0.05 vs control (Student–Newman–Keuls test). [b]p < 0.05 vs control (Mann-Whitney test). [c]Corresponds to 1800 seconds observation; Control n = 07; Ligature n = 07. [d]Corresponds to 600 seconds recording; Control n = 07; Ligature n = 07. [e]Results expressed as medians (interquartile range). [f]Results expressed as mean ± standard error of the mean

Table 2. Radiographic analysis of the mesial side of the first lower left molar of rats with distance of the cementoenamel junction to the alveolar bone crest of the control and ligature groups. Values represent mean ± standard deviation and are expressed in pixels

Groups	Radiographic analysis
Control	64.24±1.31
Control +Ligature	80.15±2.86*

** Statistically significant within the same parameter, with p <0.01 (Student's t test)*

4. DISCUSSION

This study investigated the effect of periodontal disease on the sexual behaviour of adult male Wistar rats. It was found that this disease decreased the sexual arousal of the rats, as indicated by an increase in the latency of intrusion. In addition, the intrusion frequency (erectile component) was decreased in the control group. Thus, bearing in mind that this study demonstrated that the experimental group had changes in various parameters of sexual behaviour, this indicates a possible erectile dysfunction associated with periodontitis.

Although several vasodilators have been implicated in affecting erectile response, nitric oxide appears to be the major stimulator of cavernosal vasodilation and penile erection [14-16]. An intact endothelium usually releases this potent vasodilator, which maintains vascular reactivity. However, levels of nitric oxide decrease when there are endothelial lesions, and the vasculature is therefore less reactive. Consequently, it is reasonable to postulate that any substance that reduces relaxation mechanisms or increases the contractile mechanisms of the cells of the smooth cavernous muscle may be a causative agent of erectile dysfunction. In this regard, various cytokines, particularly tumor necrosis factor-alpha (TNF-α), appear as the most important candidate in the pathophysiology of erectile dysfunction, due to its known effects on the vasculature. Studies have shown that there is a common pathogenesis for both erectile dysfunction and for endothelial dysfunction, and when the latter is present in the penis it strongly contributes to erectile dysfunction [17-20]. Likewise, Montorsi et al. [21] and Kirby et al. [22] have shown that erectile dysfunction can be the result of impaired endothelial function, as well as occlusion of the cavernous arteries by atherosclerosis.

Chronic periodontitis may contribute to the development of endothelial dysfunction, and there are three possible mechanisms to explain how this occurs. Firstly, because chronic inflammation is associated with an increase in reactive oxygen species [23,24]. The excess production of reactive oxygen species leads to an increase in the inactivation of nitric oxide (NO) and damage to the antioxidant system may contribute to endothelial dysfunction in patients with periodontitis [25].

Secondly, high concentrations of inflammatory mediators such as TNF-α, interleukin-6 (IL-6), IL-8 and IL-18 may be associated with an increased risk of endothelial dysfunction [26,27] because the concentrations of these inflammatory markers are also increased in patients with chronic periodontitis [28]. Thirdly, periodontal pathogens, or their products, may directly affect endothelial function [29]. Therefore, periodontal disease can be considered a risk factor in relation to erectile dysfunction because endothelial dysfunction is a key event in the pathophysiology of erectile dysfunction, corroborating the results presented in this study (Table 1).

The radiographic analysis can be used for diagnosing the alveolar bone height, because by measuring the distance from the cementoenamel junction to the alveolar bone crest it is possible to estimate whether there is loss or not [30]. The measurement of this distance was significantly greater in the ligature group, so it is clear that alveolar bone loss was induced by the use of the ligature. The results strengthened this hypothesis, since this measurement was also higher in the ligature group (Table 2).

Although there are few studies in the literature that examine the sexual behaviour of rats with periodontal disease, research such as that conducted by Eltas et al. [31] used a questionnaire to diagnose erectile dysfunction (The International Index of Erectile Function Questionnaire), and also investigated the sexual behaviour of individuals. In this questionnaire, men answered questions regarding their erectile function, satisfaction with sexual intercourse, evaluation of orgasmic function and sexual desire in order to evaluate the effect of periodontal therapy on the severity of erectile dysfunction in patients with chronic periodontitis.

It was revealed that the severity of erectile dysfunction improved with periodontal treatment. At the beginning of the study the treatment group consisted of 32 individuals with severe erectile dysfunction and 28 with moderate erectile dysfunction; after 3 months, 25, 29 and 6 individuals had severe, mild and moderate erectile dysfunction, respectively.

Several other studies in the literature demonstrate the relationship between periodontal disease and erectile dysfunction [9,32-35]. Among them, is a study of rats by Zuo et al. [35], which reported that a decrease in the expression of endothelial nitric oxide synthase (eNOS) and the activity of the enzyme nitric oxide synthase (NOS) in the cavernous tissue of the penis caused by the systemic inflammatory state in periodontitis may be one of the important risk factors for erectile dysfunction. The results of this particular study also showed that TNF-α concentrations in rats with periodontitis were significantly higher than in the control group. One of the roles of TNF-α is the inhibition of expression of eNOS in endothelial cells.

Although the penile vascular layers, which are responsible for erection of the penis, are far from the source of inflammation in individuals suffering from periodontitis, the endothelium is in direct contact with circulating blood and can still suffer from systemic oxidative stress. Furthermore, even if low concentrations of inflammatory cytokines were induced by oxidative stress and periodontitis, this can reach the penile endothelium, so the prognosis of endothelial dysfunction is cumulative [36] as the chronic nature of periodontitis. Therefore, the loss of the functional integrity of the endothelium and subsequent endothelial dysfunction plays a key role in the occurrence of erectile dysfunction [37].

The results of a study by Oguz et al. [33] reinforce the idea that chronic periodontitis is more often present in patients with erectile dysfunction than in those without it. In addition, the higher level of bleeding on probing seen in the group with erectile dysfunction compared with the control group, and the correlation between bleeding on probing and erectile dysfunction, supports earlier reports that a high level of infection results in erectile dysfunction due to endothelial dysfunction. Coincidentally, Zadik et al. [9] reported that chronic periodontitis was significantly more prevalent among men with erectile dysfunction compared to men without erectile dysfunction, and they suggested that this may be associated with periodontitis.

A recent retrospective case-control study by Keller et al. [32] analyzed data from 32,856 patients with erectile dysfunction and 162,480 control patients in order to evaluate the association between erectile dysfunction and chronic periodontitis. They found that the patients with erectile dysfunction were 3.35 times more likely than the controls to have been previously diagnosed with periodontitis. Another study by Sharma et al. [34] concluded that the prevalence of chronic periodontitis in patients with erectile dysfunction was higher for men who had severe erectile dysfunction (81.8%).

5. CONCLUSION

Thus, within the limits of our experimental study, it can be concluded that experimental periodontitis may contribute to a reduction in the sexual behaviour of male rats.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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