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Original Research Article

Ligature-induced periodontitis associated to alcohol using and stress on animal's behavior: study in rats

Tereza Aparecida Delle Vedove Semenoff[1]
Álvaro Henrique Borges[1]
Alessandra Nogueira Porto[1]
Fabio Luis Miranda Pedro[1]
Igor Francisco Arruda de Abreu[2]
Natalino Francisco da Silva[3]
Alex Semenoff Segundo[1]

Corresponding author:
Alex Semenoff Segundo
Rua Profa. Azélia de Melo, n. 318, apto. 63, B. Araés
CEP 78005-700 – Cuiabá – MT – Brasil
E-mail: semenoff@uol.com.br

[1] Professor of the Master in Integrated Dentistry, University of Cuiabá, Faculty of Dentistry – Cuiabá – MT – Brazil.
[2] Dentist. Trainee in the Discipline of Hospital Dentistry, University of Cuiabá – Cuiabá – MT – Brazil.
[3] Undergraduate student in Biology, University of Cuiabá – Cuiabá – MT – Brazil.

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Abstract

Introduction and Objective: To evaluate the effect of ligature-induced periodontitis association with chronic use of alcohol and stress on the behavior of the animals. **Material and methods:** Forty female rats were distributed equally into the following groups: control (CG), alcohol+stress+ligature (ASLG), stress+ligature (SLG), alcohol+ligature (ALG), ligature (LG). In the first day, the animals of ALG and ASLG were exposed to the ingestion of water and 20% alcohol solution (v/v). The animals of ASLG and SLG underwent stress testing through physical restraint for 4 daily hours, during 60 days until their euthanasia. In the next day after the beginning of the research, the animals of ALG, ASLG, SLG and LG were submitted to ligature installation around the right maxillary second molar. The evaluation of the behavior of the animals was executed through open field variables (amount of central and peripheral segments flown, times in which the animal was kept on two paws without support, self-cleaning movement - itchy nose)

and maze cross parameters (amount of entrances in the arms and time of permanence in these sites). Data were gathered, their means calculated and submitted to analysis of variance and Ducan, Kruskal-Wallis and Mann-Whitney tests ($p < 0.05$). **Results:** The results did not demonstrate statistical differences for the parameters analyzed, except from the closed filed, in which ASLG and SLG exhibited the longest time of permanence. **Conclusion:** Considering the methodology used, it was possible to observe that the alcohol associated with chronic stress and ligature-induced periodontitis demonstrated few alterations on the behavior of the animals.

Introduction

In many times, the stress of the modern life can be considered an ally in surpassing the challenges of daily life. However, for long periods and at high doses, it can cause irreversible damages to physical and mental health of an individual [3, 16]. The stress has a strict relationship with the immune, nervous and endocrine systems and it can cause from simple body pain to serious, problematic and irreversible conditions such as heart diseases, cancer and autoimmune diseases [3, 11].

Other chemical factor of organic deterioration is alcohol use. In Brazil there have been reports on the increase of the numbers of consumers and the high mortality associated with alcoholism [5]. In an epidemiologic study conducted in Rio de Janeiro, it was possible to observe a prevalence of 51% for alcohol consumption and 3% of addiction to this substance [1]. In Cuiabá, these rates are even greater, because 71% of the young interviewed affirmed they had consumed alcohol and 13.4% seemed to be alcoholics [21].

The oral diseases affect great part of the world population [22]. Periodontitis compromised the quality of life indicators of human beings [12] and it is strictly related to systemic diseases, with high public costs, being considered as a public health problem [2]. It is also important emphasizing the role of the consumption of alcoholic beverages plays in the predisposition of diseases, including oral cancer [24]. Considering the associations among alcoholism, stress and periodontitis, the aim of this study was to evaluate the behavior of the association of alcohol consumption, chronic stress, and oral infection through periodontitis induction in rats.

Material and methods

This present study was approved by the Ethical Committee in Research of the General University Hospital of the University of Cuiabá (Unic), under protocol number #0307-321.

To execute the research, 40 female adult Wistar rats (*Rattus novergicus*) obtained from the Central Vivarium of Unic were selected. The animals were randomly distributed into five groups, as follows: control group (CG), alcohol associated with ligature group (ALG), alcohol associated with stress and ligature (ASLG), stress associated with ligature (SLG) ligature group (LG).

After the formation of the groups, the animals assigned to both ALG and ASLG ingested 20% ethanol solution (volume/volume) – during the study [19].

All the periodontal disease induction was executed under sedation through intramuscular injection of 0.1 ml of ketamine hydrochloride (Dopalen, Agribrands. Saúde Animal, Paulínia, SP, Brazil) associated with 0.05 ml de xylazine hydrochloride (Rompun, Bayer. Saúde Animal, São Paulo, SP, Brazil), per each 100 grams of body weight.

In the first day of the research, the animals assigned to the ALG and ASLG were anesthetized and then submitted to the ligature installation through sterile suture thread number 4-0, (Ethicon, Johnson e Johnson, São Paulo, Brazil) around the right maxillary second molar. After the ligature installation, a period of 60 days was elapsed, and then all groups were submitted to euthanasia through anesthetic excess [20].

The stress induction was performed by physical restraint of the rats assigned to ASLG and SLG during all study. The restraint was induced by the maintenance of the animals inside PVC tubes compatible with their size. This procedure lasted 4 daily hours from 6h to 18h, at mean temperature of 24°C. The period of chronic stress duration was of 59 days, starting from one day after the periodontal disease induction [4].

For this analysis, behavioral tests of maze cross and open field were carried out. The device used in open field was a circular arena with 50 cm of radius, divided by segments in two circles: a central

one comprising eight equal parts; a peripheral one, with 16 equal parts. The maze cross device was composed of four arms: two opened and two closed. The closed arms were 30 cm of height, 53 cm of length and 13 cm of width.

After each observation in the respective analysis objects, the devices were cleaned with distilled water. For the open field analysis, the number of central and peripheral segments flown, times in which the animal was kept on two paws without support, self-cleaning movement - itchy nose were considered as parameters. For the maze cross, the parameters used were the amount of entrances in the arms and time of permanence in these sites [14].

Based on the study results, data were submitted to the analysis of variance and Ducan test, and Kruskal-Wallis with Mann-Whitney test, with level of significance of 5%.

Results

Concerning to the variable *number of times in open field*, ASLG stayed more times than LG ($p < 0.05$). There were no statistically significant differences ($p > 0.05$) among CG, SLG and ALG. For the variable *number of times in closed field*, ASLG was the most frequent group ($p < 0.05$). CG, SLG, ALG and LG did not exhibit statistically significant differences among each other ($p > 0.05$). In maze cross device, the variables *times of permanence in open and closed field* did not evidence statistically significant differences among each other ($p > 0.05$). Data are seen in table I.

According to table II, for open field device, the variables *central segment* and *self-cleaning* did not show statistically significant differences among groups ($p > 0.05$). For the variable *peripheral segment*, there were a greater amount of segments flown in ALG than in LG. The other groups behaved similar among each other ($p > 0.05$). Concerning to the variable *standing up from open field*, SLG was the most frequent group. There were not significant statistically differences ($p > 0.05$) between CG and LG. SLG behaved different from ASLG and ALG ($p < 0.05$).

Table I – Evaluations of the CNS in maze cross device. Means and standard deviations

Group	N	Amount of entrances	Time of permanence	Amount of entrances	Time of permanence
		Open		Close	
CG	8	1.50 ± 1.06 ab	50.37 ± 63.03 a	1.75 ± 1.38 a	224.62 ± 67.35 a
ASLG	8	2.75 ± 1.48 b	49.25 ± 30.12 a	5.25 ± 3.10 b	218.25 ± 40.68 a
SLG	8	1.75 ± 1.38 ab	32.50 ± 21.04 a	3.00 ± 2.67 a	252.50 ± 50.00 a
ALG	8	1.37 ± 1.40 ab	36.87 ± 50.91 a	1.50 ± 1.06 a	249.37 ± 57.22 a
LG	8	1.00 ± 0.92 a	27.50 ± 29.39 a	1.62 ± 0.74 a	258.00 ± 43.20 a

* Different letters column-wise mean statistically significant differences among groups ($p < 0.05$)

Table II – Evaluations of the CNS in open field. Means and standard deviations

Group	N	Central segment	Peripheral segment	Self-cleaning	On two paws
CG	8	3.12 ± 3.52 a	62.00 ± 27.00 a,b	1.00 ± 0.75 a	3.12 ± 4.64 a,b
ASLG	8	4.25 ± 2.65 a	65.12 ± 25.78 a,b	2.00 ± 1.19 a	0.75 ± 0.88 a
SLG	8	4.37 ± 3.99 a	55.50 ± 23.52 a,b	1.62 ± 1.50 a	5.50 ± 4.89 b
ALG	8	3.12 ± 2.79 a	73.75 ± 11.86 b	1.12 ± 0.99 a	1.37 ± 2.13 a
LG	8	5.50 ± 3.11 a	47.50 ± 16.05 a	1.25 ± 1.16 a	2.00 ± 2.82 a,b

* Different letters column-wise mean statistically significant differences among groups ($p < 0.05$)

Discussion

Under these experimental conditions, the study demonstrated that the chronic use of alcohol associated with stress and ligature-induced periodontitis was capable of providing small alterations in the behavior of the animals. The alcoholism for longer periods causes several biochemical and physiological

abnormalities in the human body [9] and it is also a contributing factor in the induction of the diseases such as cancer [13] and periodontitis [15].

Additionally to alcoholism, emotional diseases have been increasingly more frequent in modern life and they have intensified the decrease of quality of life [3, 25]. This health neglect due to stress, mainly in large cities, has intensified the emotional and physical problems, injuring the immune system [3]. The study results evidenced that the stress associated with periodontitis induction alone also caused, sometimes, a slight change in the behavior of the animals. The association of these two factors occurred according to the results of this study, which corroborates with other findings previously reported [17, 18, 23].

It could be hypothesized that there would be results with greater differences among the variables. The chronic use of alcohol and stress could have caused the adaptation of animals in this evaluation parameter [15]. The distinguished Hans Selye advocated this situation type as adaptation phase [6]. Most times, the mammalian are resistant; however, after injury by external agents for longer times, any organ or system always decompensate. These information are proved true through the evaluation of the periodontal structures in histological findings. In these groups, there had been a greater destruction of loose conjunctive tissue, bone conjunctive tissue and epithelial tissue [8]; in addition, the hematopoietic system has also exhibited variations in the same stress model [10]. It is important to emphasize that methodologies similar to the stress induction and abusive use of alcohol were capable of modifying the physiology of the animal [14, 15].

The choice for the percentage of alcohol level of this study was based on both the values found in daily market alcoholic beverages and information reported in the literature [15, 19]. The methodology of the evaluation of the behavior of the central nervous system was based on the literature [4, 7, 14], and its relationship with the dental problems starts to be explored [14]. Notwithstanding, the etiopathogeny of periodontitis has still aspects of difficult understanding and it becomes more comprehensive when the behavior and alcoholism are considered. Despite of the limitations of this study, such as the auxiliary examinations and sample size, the results were interesting. Actually, the longer time of stress and alcohol use seemed to cause the adaptation of the animals in the behavioral analyses used in this study. Further studies are necessary to achieve more consistent results regarding to the three problems of public health involved in this study.

Conclusion

Based on the methodology employed in this study, it was possible to verify that the use of alcohol associated with chronic stress and periodontitis induction demonstrated few alterations in the behavior of the animals.

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