The Effect of Periodontal Diseases and Cognitive Deficit on Behavioral State, Oxidative Stress Parameters and Alveolar Bone Loss in Rats

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Abstract

Aims: To evaluate the effect of periodontal disease (PD) and cognitive deficit (CD) on behavioral state, oxidative stress parameters, and alveolar bone loss (ABL).

Materials and Methods: Fifty male Wistar rats were randomly divided into five groups: control; scopolamine; periodontal disease (PD); PD+scopolamine evaluated by the Morris water maze behavioral test; PD+scopolamine assessed by the inhibitory avoidance behavioral test. PD and CD were, respectively, induced by ligature and scopolamine. Both the maxilla and mandible were morphometrically analyzed for ABL. For all animal groups, the study duration was one month. For the ligated animals, the final evaluation was performed 14 days after the ligature placement. Reduced glutathione (GSH), total sulfhydryl (TS), thiobarbituric acid, catalase, and superoxide essays were assessed in the brain tissues (cortex and hippocampus).

Results: Both groups with induced PD+CD (0.46±0.04mm and 0.47±0.04mm for maze and inhibitory avoidance behavioral tests, respectively) presented a significantly higher ABL in comparison to the group that received only scopolamine (0.22±0.01mm). PD and PD+scopolamine groups presented significantly lower GSH and TS in comparison to the control group. Regarding the memory latency tests, there were no statistically significant differences among groups.

Conclusion: The induction of PD was capable to modulate the CD, decreasing the action of the antioxidant agents in the brain. However, PD was not capable of impairing the short and long-term spatial memory retention behavioral.

Keywords: Periodontitis; Alzheimer's disease; Cognitive dysfunction; Wistar rats.

Introduction

Over the last 100 years, due to the progress and innovations in health care, several countries have experienced an increase of up to 30 years in human longevity and in life expectancy at birth (Ho and Hendi, 2018). Among the pathologies related to aging processes, degenerative processes stand out. Worldwide, the prevalence of these diseases, mainly related to dementia, range from 5% to 7% during the sixth decade of life (Prince *et al*., 2013). Usually, they are associated with a serious progressive morbidity (Janssen *et al*., 2019).

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Alzheimer's disease represents the most common form of cognitive deficit. It is estimated that by 2030, approximately 72 million individuals will present with this disease (Reitz *et al*., 2011). In the United States of America, the costs related to the treatment of Alzheimer's disease has reached 172 billion dollars per year (Alzheimer's Association, 2010).

The biological plausibility of the relationship between periodontal diseases and Alzheimer's disease can be explained in three ways: (i) dissemination of gram-negative bacteria from the oral cavity to the brain; (ii) neuronal injury due to transmigration of inflammatory mediators through the blood-brain barrier in response to periodontitis; (iii) genetic polymorphisms associated with the pathogenesis of both periodontal diseases and Alzheimer's disease (Singhrao *et al*., 2014; Harris and Harris, 2015; Kamer *et al*., 2015; Olsen and Singhrao, 2015; Singhrao *et al*., 2015; Ide *et al*., 2016; Ganesh *et al*., 2017).

Periodontitis is an inflammatory disease triggered by the accumulation of oral biofilms. In addition to the pro-inflammatory cytokines associated with periodontitis, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, there is also an increase in the presence of reactive oxygen species (ROS) (Battino *et al*., 1999; D'aiuto *et al*., 2010). The imbalance between ROS production and the antioxidant defenses leads to oxidative stress (D'aiuto *et al*., 2010; Callaway and Jiang, 2015; White *et al*., 2016), which may result in cellular and tissue damage.

Oxidative stress, through the action of free radicals, causes cellular damage via lipid peroxidation, protein and DNA oxidation (Akalin *et al*., 2007; Konopka *et al*., 2007; Baltaciğlu *et al*., 2008; Canakci *et al*., 2009; Villa-Correa *et al*., 2015). In order to prevent the destructive actions of free radical cytotoxic, several antioxidant mechanisms exist at the molecular level, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) (Cui *et al*., 2004).

Oxidative stress may play an important role in Alzheimer's disease (Ricciarelli *et al*., 2007). Additionally, the expression of antioxidant enzymes may be important agents in the in the central nervous system and in peripheral tissues of patients with Alzheimer's disease (Chen and Zhong, 2014). Therefore, the aim of the present study was to evaluate the effect of periodontal disease (PD) and cognitive deficit (CD) on the behavioral state, oxidative stress parameters and alveolar bone loss (ABL) in rats. The hypothesis of this study is that the inflammatory process in periodontal tissues influences the development and progression of CD in Alzheimer's disease with the involvement of oxidative stress induction.

Materials and Methods

Experimental design and ethical aspects

This study followed the ARRIVE (Animal Research: Reporting In Vivo Experiments) guideline for animal researches (Kilkenny *et al*., 2012). The study protocol was approved by the Animal Research Ethics Committee of the Universidade Regional Integrada do Alto Uruguai e das Missões (CEUA-URI/Erechim) under protocol #051. The experimental procedures followed the protocol proposed by the Universal Declaration of Animal Rights (UNESCO - January 27, 1978) and the International Ethical Guidelines for Biomedical Research Involving Animals (Council for International Organizations of Medical Sciences - CIOMS) for animal research.

Animal model

Fifty male Wistar rats, aged 45 days, weighing approximately 250g were used. During the experimental period, animals were housed in polypropylene boxes, measuring 65x25x15 cm, with four to five animals in each box. Room conditions included 12/12h light and dark cycles, 55% humidity, and 22±2°C temperature. Moreover, food and water were available *ad libitum*.

Efforts to minimize pain and discomfort were made by the researchers, and the rats were acclimated one week prior the beginning of the experiment. Sample size estimation was based on previously published behavioral analysis studies (Eun *et al*., 2017) and alveolar bone loss (ABL) (Liberman *et al*., 2011) as the main outcomes. Accordingly ten animals per group were used. A posteriori analysis was performed in order to calculate the power of the present study. It used the mean and standard deviation of alveolar bone loss in both PD and Scopolamin groups. When a 95% confidence interval was established, a power analysis of 100% was demonstrated.

Randomization and animals allocation

The five experimental groups were determined by weight-stratified randomization, using the quartiles of weight. The experimental groups were: Control Group – no induction of both ABL and CD (n=10); Periodontal disease group – only ABL was ligature-induced (n=10); Scopolamine Group – only CD was induced by scopolamine, and behavioral analyses are made by Morris water maze and inhibitory avoidance (n=10); Scopolamine + PD (Morris water maze) Group – both ABL and CD were induced by ligatures and scopolamine, respectively, and the Morris water maze behavioral test was performed (n=10); Scopolamine + PD (inhibitory avoidance) Group – both ABL and CD were induced by ligatures and scopolamine, respectively, and the

inhibitory avoidance behavioral test was performed (n=10). For all animal groups, the study duration was one month. For the ligated animals, the final evaluation was performed 14 days after the ligature placement.

Ligature placement

Ligatures were placed under general anesthesia. The anesthetic protocol included Tiletamine-Zolazepan (Zoletil®, Virbac Brasil, São Paulo, Brazil), 20mg/kg, intraperitoneal injection, with supplementation as needed (Diehl *et al*., 2001). ABL was induced by placing four ligatures around all second molars in both the upper and lower arches, using a black silk strand (4-0) (Ethicon®, Johnson & Johnson, New Jersey, USA). Ligatures were placed by an experienced researcher, and the knots were placed in the buccal surfaces. The protocol proposed by Liberman *et al*. (2011), with ligatures remaining for 14 days in order to induce periodontitis was used. The presence and correct position of the ligatures were verified at the end of the study to ensure the challenge was in place.

Cognitive deficit induction

Memory deficit was induced by the intraperitoneal administration of hydrobromide scopolamine (3mg/kg) (Sigma, São Paulo, Brazil). This administration was performed 30 minutes prior Morris's inhibitory avoidance and water maze tests (Bihaqi *et al*., 2011).

Brain tissue preparation and oxidative stress measurement

Animals were euthanized by decapitation, without the use of any anesthetics, to prevent alterations in the brain biochemical analysis. Immediately after euthanasia, the cerebral cortex was separated. The structures were homogenized (1:10 p/v) in a sodium phosphate buffer (200 mM) with KCl (140 nM), which were centrifuged in 3,000 rpm during 10 minutes. The supernatant was separated for the homeostasis redox techniques, and the samples were incubated in 37°C in the presence of organic acids for one hour.

Determination of the thiobarbituric acid-reactive substances assay

In order to quantify the final lipid peroxidation products, thiobarbituric acid-reactive substances (TBA-RS) method were used. 300 μL of 10% thiobarbituric acid in a 150 μL aliquot of the supernatant was added. After centrifugation, 300 μL of the supernatant was treated with 0.67% of thiobarbituric acid in a 1:1 proportion. The mixture was taken to a boiling bath for 25 min and then cooled in water at room temperature. The absorbance obtained by the resultant pink color was measured using a spectrophotometer at 532 nm (Delwing-Dal Magro *et al*., 2016)

Measurement of total sulfhydryl content

To determine the total thiols in the samples, total sulfhydryl content was determined (Aksenov and Markesery 2001) in order. A total of 50μL of the supernatant reacted with 10mM of dithionitrobenzoic acid (DTNB). Oxidation of the free thiols in the sample leads to disulfide bond formation; the DTNB is reduced by the unoxidized thiols, generating a yellow derivative that was read spectrophotometrically at 412 nm.

Measurement of reduced glutathione

This essay was performed accordingly to the Salat *et al*. (2014) method. A volume of 300 μL of the supernatant was deprotected by adding a volume of 1.85% metaphosphoric acid. After centrifugation, 150 μL of supernatant was neutralized with Na_2HPO_4 (0.3 M) and then reacted with DTNB in a concentration of 1mM. The measurement of free thiols from a deproteinized sample reflects the reduced glutathione (GSH) concentration of the sample.

Catalase activity

Catalase activity was determined by the method of Olorunnisola *et al* (2016). In 50mL buffer of 10mM potassium phosphate, pH 7.0, 100μL of 30% H_2O_2 (v/v) was added. Subsequently, 20μL of the supernatant (previously treated with Triton 0.1%) was mixed with 600μL of the mixture (buffer + H_2O_2). The H_2O_2 absorbance was analyzed in a spectrophotometric of 240nm.

Superoxide dismutase enzyme activity

Superoxide dismutase activity was measured as described by Marklund (1985). The supernatant obtained after the centrifugation (15μL) was transferred, 96 well plates. The superoxide dismutase (SOD) activity was measured using the auto oxidize capacity of the pyrogallol in a process highly dependent of O^{2-}, which is a SOD substrate. The auto oxidation inhibition of this compound occurs in the presence of SOD, and its activity can be indirectly measured by pectrophotometric measurement at 240nm. This process was carried out in duplicate. Subsequently, 210μL of a mixture of 80U/mL (100μL) of catalase and 50nM Tris buffer, ethylenediaminetetraacetic acid (21500μL), pH 8.2, was added to each well. Lastly, 20μL pyrogallol (0.38 mM) was added. The spectrophotometric absorbance reading at 405 nm was taken after 5 min. A calibration curve was made with purified SOD as standard to calculate the SOD activity present in the samples. The results are expressed as μmol/mg.

Inhibitory avoidance behavioral test

The inhibitory avoidance behavioral test was performed by evaluation of the short- and long-term aversive memory evaluation. This test was performed in a conditioning box with a front glass surface. A wood platform,

measuring 8cm x 25cm, was located in the left of the box and 5 cm of the floor of the box. The remaining parts of the box were composed of stainless steel grids, which presented 0,2 cm of diameter and separated by one another for a space of 1 cm.

In the training session, that occurred before ligation the animals were gently placed facing the bottom of the box, and when the four legs were on the stainless steel grids, they received an electrical stimulus of 0.4 mA, for 2 seconds, and the descent latency was recorded. After that, the animals were removed from the equipment and placed in their boxes. Two test sessions were performed, the short-duration memory test happened 90 minutes after the training session, meanwhile the long-duration memory test happened 24 hours after the training sessions. In both test sessions, animals were exposed again to the equipment, but without an electric stimulus. The difference between the descent time (latency) during the test session and the training session was recorded as the measurement of memory.

In order to standardize, a maximum time of latency during the training sessions of 60s was established and, during the test session, a maximum of 180s was established to all experimental groups (Rossato *et al*., 2004). Tests were performed after 14 days of ligature placement, when experimental periodontitis was present. In all groups, tests were performed after 30 minutes of administration of scopolamine.

Morris Water Maze Test

In this test, a tank (1m of diameter and 30cm of depth), divided into quadrants (north, south, east, and west) by imaginary lines, containing water was used. During the training, a platform (10 cm of diameter) was placed 1cm underwater (21±2°C). The water was dyed with opaque water turbidity solution. For a period of four consecutive days, the animals were placed in the same quadrant, preceding the administration of scopolamine. Each training consisted of four tries, during which the animals were gently placed in one of the quadrants for a maximum of one minute each. If the animals did not locate the platform within 60 seconds, they were guided by the researcher to the platform, and kept on it for 10 seconds, then, returned to their box and rested for 20 seconds. For this test, the time that animal took to reach the platform was recorded on the fourth day. On the fifth day, the animals received the scopolamine administration, and the test was performed.

The test consisted of repeating what they had learned in the training sessions, but without the platform in the water. The animals were allocated at a starting point and were released to swim in the position of the pool opposite to the platform, allowing them to evaluate the spatial memory (Rossato *et al*., 2006). Comparisons between the different groups were performed. This test was also performed 14 days after the ligature-induction periodontitis.

Preparation of the Jaws for the Morphometric Analysis of alveolar bone loss

Following euthanasia, both the maxilla and mandible were removed and immersed in sodium hypochlorite (9% active chlorine), for five hours, and the soft tissue were mechanically removed. Afterwards, the samples were washed and dried. For a better visualization of the cement-enamel junction, the pieces were stained with 1% methylene blue for one minute. The pieces were washed and dried again to remove excess solution. Standardized photographs were taken of the pieces using a professional camera (model D100, 6.1 megapixels, with 100 macro lens - Nikon®Coolpix, Ayutthaya, Thailand). The camera was placed in tripod and remained in a standard focal distance.

An apparatus made of heavy silicon paste was used to fix an endodontic ruler at a perpendicular position to the floor. The pieces were fixed to the endodontic ruler with 07 wax, so that the occlusal plane of the piece was parallel to the floor. For each piece, the buccal and lingual aspects were photographed. The distance from the cemento-enamel junction to the bone crest was measured by the Image J software (National Institutes of Health, USA). For each photograph, five measurements (two in the mesial root, one in the furcation, and two in the distal root) were performed by a blinded and calibrated examiner. The mean of the alveolar bone loss (ABL) was recorded for each animal.

Blinding and examiner reproducibility

The examiners that performed both the morphometric and behavioral analyses were blinded to group allocation. Prior to the morphometric analysis, 20 pieces were randomly chosen, and the ABL of these pieces were measured twice by the same examiner within one-week interval. The intra-class correlation (ICC) showed a coefficient of 0.97 for ABL.

Statistical analysis

All analyses were performed by the software SPSS version 22.0 for Windows (Statistics IBM®, College Station, TX, USA). In order to test data normality, the Kolmorov-Smirnov test was performed. For the comparison of mean ABL and oxidative stress parameters among groups, the ANOVA test for multiple comparisons was applied. Regarding the mean latencies between groups in the behavioral analysis, it was used t-test for independent samples. Mean and standard deviation are presented to all the variables. The level of significance adopted was $\alpha<5\%$.

Results

From the 50 animals that commenced the study, 47 completed all the experimental procedures. During ligature placement, three animals died due to complications in the anesthetic recovery, one in the PD group, one in the scopolamine group, and another from Scopolamine + PD (inhibitory avoidance) group.

Figure 1 shows the comparison among groups for ABL. It was observed a statistically significant difference among groups, as PD group (0.48±0.04 mm), Scopolamine + PD (inhibitory avoidance) group (0.46±0.04 mm), and Scopolamine + PD (Morris water maze) group (0.47±0.04 mm) presented a significantly higher ABL when compared to the control (0.18±0.03 mm) and scopolamine (0.22±0.01 mm) groups.

Figures 2a to 2e demonstrate the mean difference of the oxidative stress parameters, in the brain tissues, among the different groups. In these analyses, significantly lower levels of GSH and total sulfhydryl were detected in PD (GSH: 5.15±0.80 and total sulfhydryl: 48.14±6.17) and PD + scopolamine groups (GSH: 3.87±0.72 and total sulfhydryl: 42.00±6.25) in comparison to control group (GSH: 8.00±0.77 and total sulfhydryl: 62.59±12.05). As both groups that received PD and CD inductions presented similar results of the oxidative stress parameters, they are didactically presented as one group (PD + scopolamine).

Figures 3 and 4 show the comparisons between scopolamine and PD + scopolamine groups for the Morris water maze and inhibitory avoidance behavioral test, respectively. In both behavioral tests, no statistically significant difference was observed among groups.

Discussion

The present study aimed to evaluate the effect of ligature induced-periodontitis in the cognitive deficit by two complimentary evaluations, the oxidative stress parameters in the brain tissue and the behavioral analyses for the memory of short- and long-terms. There have been some recent reports in the literature concerning the capacity of periodontitis in modulate other neurodegenerative diseases, such as Alzheimer's disease (Wu *et al.*, 2016; Tonsekar *et al.*, 2017). Epidemiological data demonstrated several factors for the biological plausibility between PD and Alzheimer's disease. Therefore, further studies are warranted in order to understand the mechanisms that associated both diseases, particularly the contribution of oxidative stress that has been related to these diseases (D'aiuto, 2010; Kanzaki *et al.*, 2017).

Animal model studies, despite their impossibility of direct extrapolation to humans, are feasible and relevant alternatives to study the interrelationship between periodontal pathologies and other systemic adverse effects (Weinberg and Bral, 1999; Oz *et al.*, 2011; Vargas-Sanchez *et al.*, 2017). To support such studies it is noted that there are a number of anatomical, biochemical and, partly, microbiological similarities in the periodontal tissues and in disease pathogenesis between some animals and humans. It is important to highlight that ligature induced-periodontitis was induced by placing four ligatures (in all second molars in each hemiarch), which increases the inflammatory challenge, mimicking the profile of generalized periodontitis in humans, unlike other studies using only one or two ligatures (Verzeletti *et al.*, 2012; Wara-Mihada *et al.*, 2017). The induction of CD by scopolamine demonstrated no significant reduction

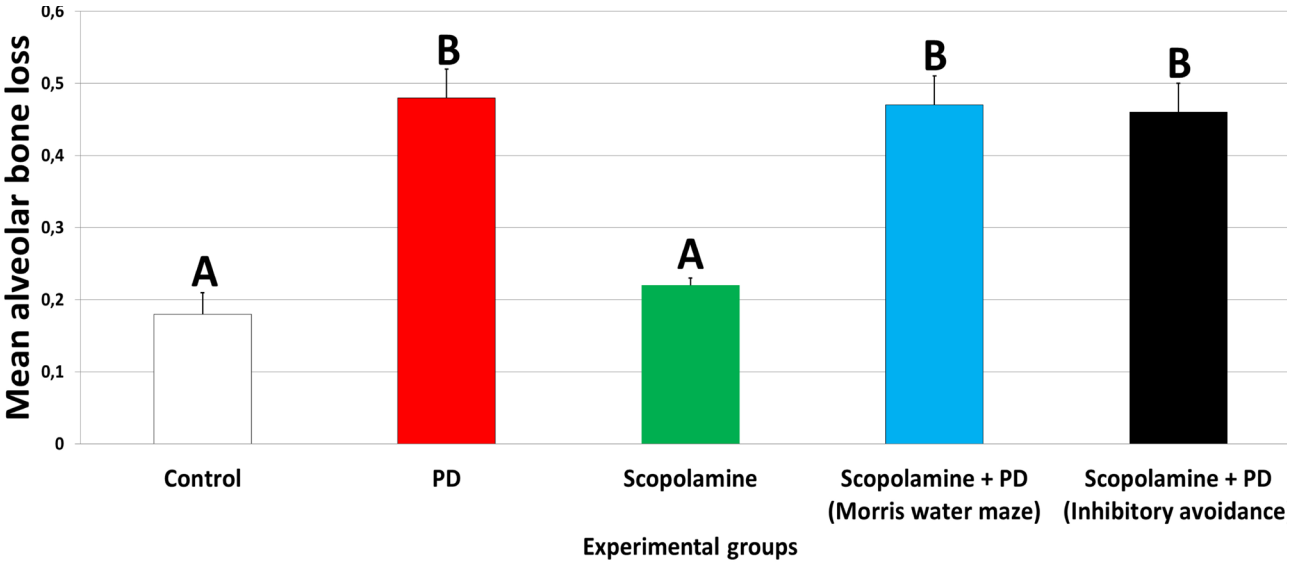

Figure 1. Comparison among the experimental groups for the mean alveolar bone loss (in millimeters). Different letters indicate statistically significant differences by one-way ANOVA with post-hoc Bonferroni (p<0.05).

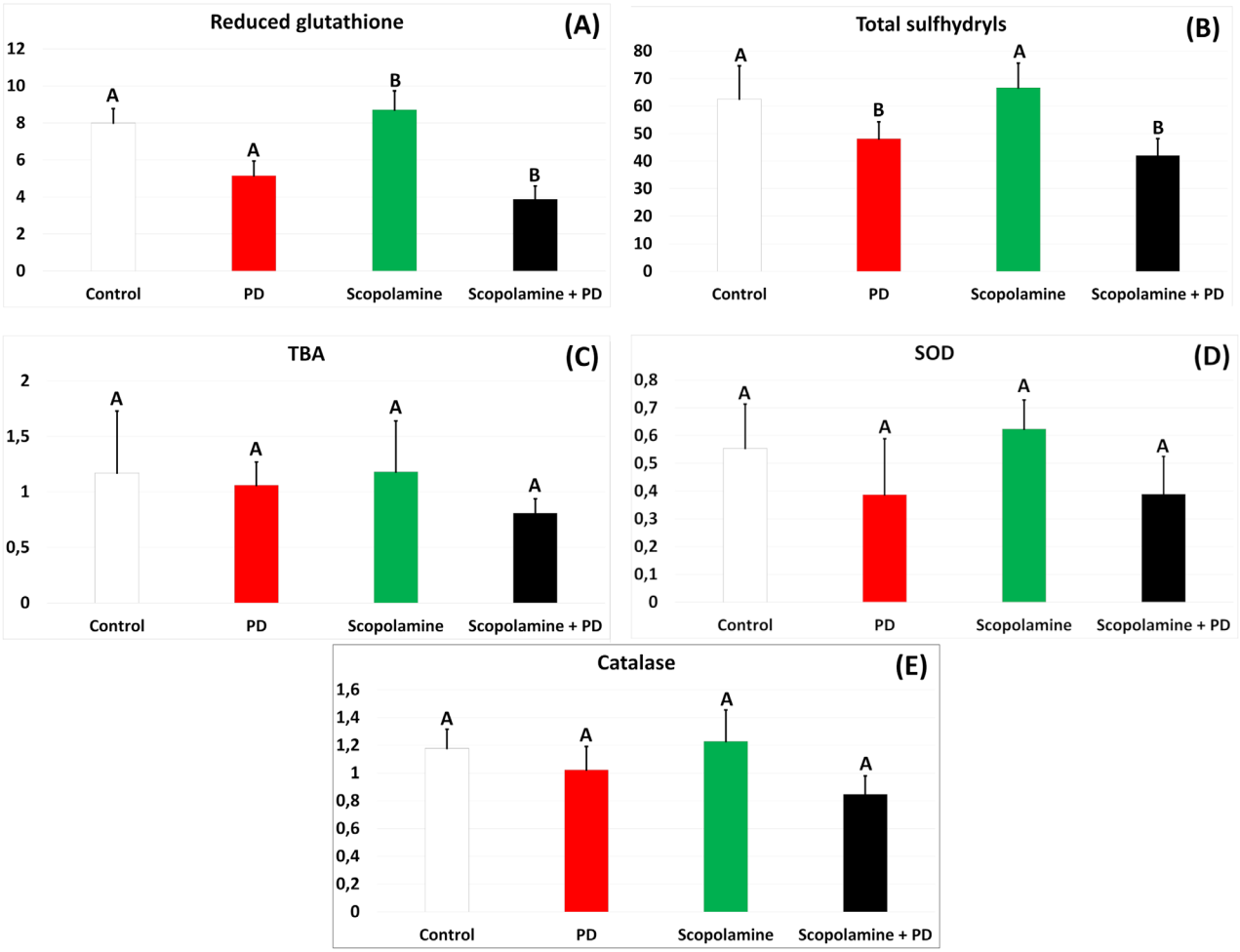

Figure 2. Comparison among groups for the oxidative stress parameters (A: Reduced Glutathione; B: Total sulfhydryls; C: Thiobarbituric acid-reactive substance; D: Superoxide dismutase; E: Catalase). Different letters mean statistically significant differences by one-way ANOVA with post-hoc Bonferroni (p<0.05). Values represent means ± standard deviation expressed as nmol GSH/mg protein (A), nmol sulfhydryl/mg protein (B), nmol TBA/mg protein (C), unit/mg protein (D and E).

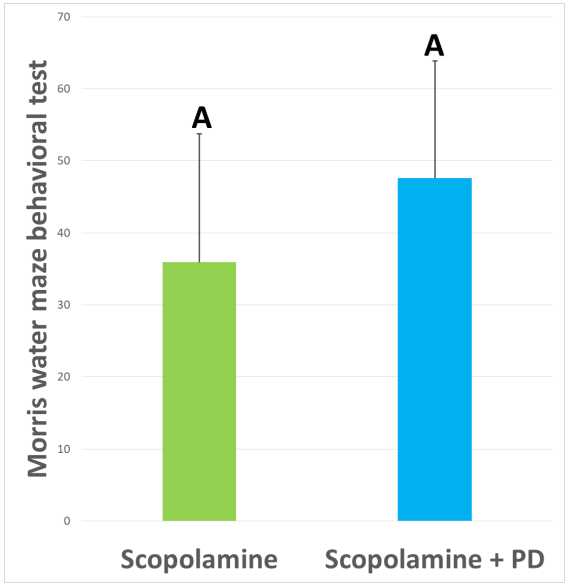

Figure 3. Comparison between periodontal disease and periodontal disease + Scopolamine groups for the Morris water maze behavioral test. Different letters indicate statistically significant differences by t-test for independent samples (p<0.05).

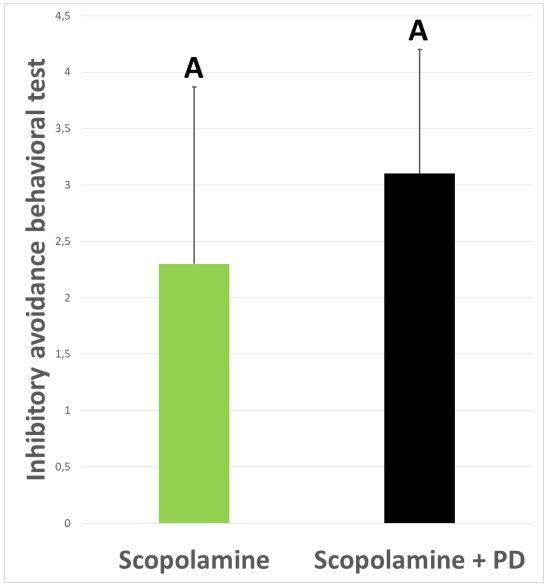

Figure 4. Comparison between periodontal disease and periodontal disease + Scopolamine groups for the inhibitory avoidance behavioral test. Different letters indicate statistically significant differences by t-test for independent samples (p<0.05).

in the antioxidant agents when compared to the control group. This results contrast with other studies in the literature (Jawaid *et al.*, 2015; Pattanashetti *et al.*, 2017), which may be explained by the different experimental model, different dosages of scopolamine, variations in the animal species, age, and complex biochemical compensatory mechanisms of the combined action of antioxidants (Chapple and Mathews, 2007).

Studies have demonstrated that periodontitis, mainly in the severe stages, has a potential to trigger a systemic pro-inflammatory and pro-oxidative stress stage, which reduces the antioxidant capacity (D'aiuto, 2010; Kanzaki *et al.*, 2017). In the same way, the pathogenesis of the Alzheimer's disease may be modulated by imbalance of oxidative factors and antioxidant action (Cervellati *et al.*, 2016). Furthermore, experimental animal models of the development of CD by scopolamine and behavioral analysis of memory, related to pathogenesis of Alzheimer's disease, are used to evaluate the retention of short- and long-term spatial memory (Malin *et al.*, 2015; Maleki *et al.*, 2017; Li *et al.*, 2017).

In the present study, ligature induced-periodontitis alone was able to significantly decrease the levels of GSH antioxidant agents and total sulfhydryl in comparison to the control group. Moreover, it was demonstrated that ligature-induced periodontitis associated with scopolamine was able to significantly decrease the antioxidant agents (GSH and sulfhydryl), when compared with only scopolamine or with control. These data suggest that ligature induced-periodontitis alone was able to increase oxidative stress, and when associated with the induction of CD potentially modulates and decreases the action of the antioxidant agents (GSH and sulfhydryl). Regarding the oxidative stress parameters, it is recommended to assess both enzymatic (SOD and catalase activities) and nonenzymatic (GSH) antioxidant defenses, as well as lipid (TBA-RS) and protein (sulfhydryl) oxidative damage parameters (Nibali and Donos, 2013).

In the present study, significant alterations in GSH and sulfhydryl levels were observed, however no significant changes in the other oxidative stress parameters (Catalase, TBA, and SOD). These findings may not be interpreted *per se*, as the measurement of a specific antioxidant does not take into account the action of all antioxidants. Therefore, the sum of all individuals antioxidants may not represent the total capacity of neutralizing the oxidative stress and repairing tissue damages. Accordingly, total antioxidant levels may provide data on the complexity of interactions more reliably (Tarpey *et al.,* 2004; Chapple and Mathews, 2007). The increased levels of some antioxidants, as noted in the present study, may be explained by compensatory biochemical mechanisms, which the interactions among the antioxidant agents may determine an increase in the levels of the oxidative stress due to a local source (Bullon *et al.*, 2014). Studies that assess these parameters are very important, because the oxidant potential and the action of antioxidant agents may be involved in the early diagnosis of the Alzheimer's disease. The identification of altered levels of antioxidant agents may be future disease biomarkers (de Leeuw *et al.*, 2017; Wojsiat *et al.*, 2017).

This is the first study to evaluate a possible effect of periodontal disease in the CD induced by scopolamine, analyzing two behavioral parameters, inhibitory avoidance and Morris water maze, in Wistar rats. There is one published study reporting a relationship between periodontitis and Alzheimer's disease, that analyzed the direct colonization of periodontopathogens at the brain level (Poole *et al.*, 2015). In that study, *Porphyromonas gingivalis* appeared to have been capable of accessing the brain of the affected rats, contributing to activation of the complement system and leading to neuronal injury. However, this study did not perform a behavioral analysis in the animals.

Regarding ABL, the present study showed no significant difference between the groups of ligature induced-periodontitis only and ligature induced-periodontitis and induction of CD. This may be explained due to behavioral changes in spatial memory latency being present in more advanced stages of cognitive deficit. It was reported that the biochemical alterations, at the brain level, mainly related to oxidative stress, are present prior to any behavioral change (Liu *et al.*, 2008; Liu *et al.*, 2009). Other factors that may modulate the behavior and influence performance on memory tests are animal species, emotional stress, and age (D'hooge and Deyn, 2001; Bromley-Brits *et al.*, 2011).

The limitations of the study include the following. (1) The short-term character of the study. In the ligated animals, the ligature (for periodontitis induction) was placed 14 days prior to final evaluation. Most studies on ligature-induced periodontitis retain the ligature in place for much longer (at least twice the time used in the present study) Holzhausen *et al.*, 2004, Cetinkaya *et al.*, 2007, Fernandes *et al.*, 2007, Semenoff *et al.*, 2008, Pepelassi *et al.*, 2012, Xynogala *et al.*, 2012) prior to final evaluation. (2). The method used to assess alveolar bone loss. The histological assessment is accurate in measuring alveolar bone loss (Dumitrescu *et al.*, 2004) and is considered the gold standard. Photography under magnification was used in the present study. (3) The relatively small number of the animals. However, there is a contemporary tendency to reduce the number of animals studied in each group (Flecknell, 2002). Therefore additional information coming from a substantial increase in the number of the animals studied might be limited. (4) The study was performed in rodents. Studies with rodents are important to understand the biological plausibility and pathogenesis, but their translational potential are not straightforward. The above limitations

reveal that the findings of the present study should be interpreted with caution.

From this study it is concluded that, in the present experimental model, ligature induced-periodontitis modulated cognitive deficiency in an animal model of Alzheimer's disease and this was associated with decreasing action of antioxidant agents at the brain level. However, this model did not show any modulation of behavioral impairments oin terms of short- and long-term spatial memory retention in Wistar rats. Additional studies are warranted in order to evidence the possible association between periodontitis and cognitive deficit-related diseases, such as Alzheimer disease, mainly related to oxidative potential at the brain level.

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