THE INFLUENCE OF LOW DOSE OF DOXYCYCLINE ON INDUCED PERIODONTITIS ASSOCIATED WITH OCCLUSAL OVERLOAD

A influência de baixas doses de doxicilina sobre a periodontite induzida associada à sobrecarga oclusal

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RESUMO

A degradação dos tecidos de suporte periodontal é o principal evento biológico da doença periodontal. O objetivo deste estudo foi avaliar a influência do uso sistêmico de baixas doses de doxiciclina (BDD) sobre o suporte ósseo periodontal (SOP) e a perda de inserção (PI) na periodontite induzida associada à sobrecarga oclusal em ratas. Neste estudo foram utilizadas 25 ratas Wistar pesando 250g, divididas em 5 grupos (n=5): GC (controle), GL (ligadura); GO (sobrecarga oclusal); GLO (ligadura e sobrecarga oclusal); GLOD (ligadura sobrecarga oclusal e doxiciclina). A periodontite foi induzida pela inserção de uma ligadura ao redor do 1º molar inferior (1MI) permanecendo por 28 dias. Para que o 1MI ficasse em sobrecarga oclusal, as superfícies oclusais do 2º e 3º molares superiores foram desgastadas. Os animais do grupo GLOD receberam 0,14 mg de doxiciclina por via oral diariamente por todo o período experimental. Os animais foram sacrificados e as hemimandíbulas direitas foram analisadas por meio de radiografias digitais quanto ao SOP (%) e as hemi-mandíbulas direitas foram processadas para a análise histopatológica da PI (mm). Os valores médios foram submetidos à análise de variância (ANOVA/Tukey) ao nível de 5%. Os resultados para SOP em GC (55.49±4.86) e GO (60.03±3.37) foram iguais entre si e diferentes de GL (46.73±4.80), GLO (51.69±2.84) e GLOD (57.76±4.50) (p=0.001). Considerando a PI, GC (0.11±0.07) e GO (0.12±0.10mm) foram semelhantes entre si e diferentes de GL (0.41±0.09), GLO (0.58±0.12) e GLOD (0.54±0.12) (p=0.0001). Em conclusão, o uso de BDD pareceu não modular a redução de SOP ou PI em ratas com periodontite induzida e sobrecarga oclusal.


INTRODUCTION

Periodontal disease is a multifactor inflammatory disorder in the gingival crevice associated with increased periodontal pathogen colonization. The periodontium is functionally adaptable to resist and support the pressure of masticatory forces. Some studies have sought to identify whether smoking, occlusal overload, diabetes and other events (Kinane, 2001; Nociti et al. 2000), such as environmental and host risk factors, are involved with in the onset and progression of periodontal disease. Occlusal overload associated with periodontal disease may produce irreversible changes in periodontal structures, leading to the rupture and attachment loss of collagen fibers and increased periodontal pocket depth (Carranza 2004).

Nogueira-Filho studied an experimental model based on the occlusal trauma as a risk factor in periodontal disease and found significant changes at the periodontal ligament space in the furcation area of lower molars submitted to occlusal overload, ligature and nicotine use. The onset of periodontal disease occurs near the gingival crevice and initially involves...
the protective periodontium. This injury progresses towards the destruction of the supporting periodontium, which is characterized by bone resorption, connective attachment loss and apical migration of the junctional epithelium (Nogueira-Filho et al. 2004).

The purpose of periodontal therapy is to control bacterial infection and to control the inflammatory process using mechanical or medication treatment and directly or indirectly modulate the host response. Tetracyclines are proven antimicrobials that also present inhibition properties in relation to host matrix metalloproteinase (MMP) activity. Therefore, this medication has been used with mechanical treatment in combined therapy for periodontal disease.

Doxycycline has been well studied, since it is the tetracycline most capable of inhibiting collagenase activity (Ryan 1996). Bezerra described the inhibition of bone resorption in rats submitted to low doses of doxycycline in ligature-induced periodontitis and observed that the process was related to the dose administered. This decrease was marked by lower numbers of osteoclasts and inflammatory cells in the groups tested when compared to the control group. Histological analysis also showed the integrity of the alveolar bone crest in doxycycline-treated rats (Bezerra et al. 2002). In a review of the literature, Preshaw et al. reported that long-term usage of low doses of doxycycline were related to the improvement of clinical parameters without bacterial resistance to tetracycline. The ability of doxycycline to modulate the collagenase activity has been studied as a new protocol in periodontal therapy. Ashley also reported this modulation in a 12 week LDD therapy protocol combined with scaling and root planning mechanical therapy for periodontal disease. In this study, a reduction in collagenase activity was observed at the gingival crevice for all the treated rats after four weeks (Ashley 1999). Karimbux et al. also observed the modulation of collagenase activity on the connective insertion (Karimbux et al. 1998). A short-term administration period of LDD was sufficient to reveal the inhibitory effect in the host response at the periodontium (Bezerra et al. 2002). However it is not knew if this inhibitory effect can be effective on periodontal breakdown when this process is associated with occlusal overload. The aim of this study was to evaluate the effect of LDD on the periodontal bone support reduction and attachment loss in female rats with induced periodontitis associated to occlusal overload.

MATERIALS AND METHODS

Experimental design
This study was performed according to ethical guidelines and it was submitted and approved by the Ethics Committee in Animal Research of UNITAU under protocol number 010/2005. Twenty-five female rats (Wistar, albinus Rattus novergicus), ten weeks-old, weighing approximately 250 g each were housed in temperature-controlled rooms and received water and food ad libitum. They were randomly divided into five groups: group CG, control; group LG, ligated; group OG, presenting occlusal overload; group LOG: ligated and presenting occlusal overload; and group LODG, ligated, presenting occlusal overload and medicated with 0.14mg of doxycycline.

Periodontitis induction and occlusal overload
Periodontitis was induced by the placement of cotton thread around the first lower molar of each rat in LG and LOG groups for 28 days. To insert the ligature, the rats anesthetized with a general analgesic drug solution (xylazine hydrochloride, Anasedan, Vetbrands laboratory, Jacareí, SP, Brazil) and a muscle relaxant (ketamine base, Dopalen, Vetbrands laboratory) in a 1 to 0.5 ml ratio, using 0.1 ml per 100 g of rat body weight (Ricardo et al. 2006). To obtain occlusal overload at the first lower molar, the second and third upper maxillary molars were flattened along their occlusal surfaces without pulp exposure in OG, LOG and ODG groups.

Drug treatment
During the periodontal disease induction period daily doses of 0.14mg of doxycycline (0.7 mg/dl concentration) were orally administered to the rats from group LODG once a day.

Radiographic analysis of periodontal bone support (PBS)
After 28 days the rats were killed and the hemi-mandibles were removed and fixed in 10% formol solution. The right hemi-mandibles were submitted to radiographic analysis and the left hemi-mandibles were processed for histopathological analysis. The samples were radiographically analyzed using the Digital RVG® Intraoral Radiographic System (Radiovisiography, Trophy Radiology Inc., Marietta, GA, USA) and the images were captured with an Adopted load apparatus (CCD sensor). The radiographs were obtained with a Gendex 765DC® Intraoral X-ray Unit (Gendex, Dentsply Internacional, USA) apparatus, using 0.08 seconds of exposure time and a focal distance of thirty cm from the sensor. Points were established for periodontal bone support measurements: the most coronal part of the distal cusp, the top of the bone crest or bone defect and the distal root apex (Fig 1). These points were considered linear measurements to determine PBS (Klausen, 1989). The measurements were expressed as a percentage of the proportional values obtained.
Histological analysis of attachment loss (AL)

The samples were processed for histological analysis by decalcification in 17% EDTA solution and were then embedded in paraffin with their vestibular surface towards the cutting plane. Mesial to distal sections of 6 μm thickness were made and were stained with hematoxilin-eosin to evaluate the connective attachment loss at the distal surface of the lower first molar. The cement-enamel junction and the insertion of the first periodontal ligament fibers at the root cementum were chosen as reference points to the linear measurement of AL. Histological descriptive analysis was made in every group. Histometric and radiographic analysis were performed using the Image Tool software for images (Windows, version 2.0, UTHSCSA, San Antonio, TX, USA). All measurements were made blind to the group assignment of the rats.

Statistical analysis

The mean values of PBS and AL of groups were analyzes by variance analysis. The correlation of the variable was analyzed by the Pearson correlation test. The analysis was realized using the Biostat software version 5.0, both at a 5% significance level.
RESULTS

Descriptive histological analysis

Histological analysis of the fragments from the control group revealed the periodontium presenting a normal appearance, with a triangle papilla at the interdental space between the first and the second molars covered by stratified squamous nonkeratinized epithelium. Periodontal ligament fibers were inserted at the root cementum along its extension (Fig. 2). The furcation area of the first molar was filled with bone tissue and periodontal ligament with uniform dimensions were observed at the top of the furcation (Fig. 3). The interdental spaces from the LG, LOG and LODG groups were filled with gingival papilla in a concave shape and covered by stratified squamous nonkeratinized epithelium. In these sections the epithelium was observed apically to the cementoenamel junction and over the root cement, showing that the attachment loss of the ligament fibrils had occurred on the cementum in this region (Fig. 3). An increase in the periodontal ligament area was observed in the furcation region of the first molar in the LG group. Besides bone loss and the increase in periodontal space, some sections showed the presence of epithelium at the top of the furcation, which means that attachment loss occurred in this region in the LOG and LODG groups (Fig 4).

Radiographic and morphometric analysis

Analysis of the PBS results showed statistically significant differences among the groups studied (p = 0.001), concerning LG as de lowest PBS and OG the highest. Other groups showed themselves with similar mean PBS values. Analysis of the AL results also showed statistically significant differences among the groups (p = 0.0001), and homogeneous group formation was observed in two groups with similar mean values in which was not inserted the ligature (Tab 1). PBS and AL revealed a weak negative correlation between themselves (Fig 5).

DISCUSSION

The modulator effect of LDD on alveolar bone support reduction and connective attachment loss that occurs in periodontitis induced by ligature procedure associated with occlusal overload in female rats was the main objective of this study. The use of animal models has been an important recourse to control the confounding variables normally presented in human studies. Klausen B has already commented about the importance of overcoming limitations, difficulties and interference factors in human studies. Some authors have also affirmed that the use of rats in experimental studies is favorable to evaluate the influence of certain risk factors concerning the onset and progression of the periodontal disease (Nociti et al. 2001; Benatti et al. 2003; Nociti et al. 2004). Klausen observed that the use of animals of similar ages was an important detail for controlling confounding factors when the cement-enamel junction and alveolar bone crest are considered as anatomic references (Klausen, 1991). Based on these statements the present study considered these criteria and the rats presented similar age and weigh for all the experimental groups.

The induction of periodontitis by the technique of ligature placement has been used in studies on rats by numerous

<table>
<thead>
<tr>
<th>PBS (%) Mean / SD</th>
<th>AL (mm) Mean / SD</th>
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<tr>
<td>GC 55.49 ± 4.86</td>
<td>BC 0.11 ± 0.07 A</td>
</tr>
<tr>
<td>GL 46.73 ± 4.80</td>
<td>A 0.41 ± 0.09 B</td>
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<tr>
<td>GO 60.03 ± 3.37</td>
<td>C 0.12 ± 0.10 A</td>
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<tr>
<td>GLO 51.69 ± 2.84</td>
<td>AB 0.58 ± 0.12 B</td>
</tr>
<tr>
<td>GLOD 57.76 ± 4.50</td>
<td>BC 0.54 ± 0.12 B</td>
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<td>p 0.001</td>
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PBS= periodontal bone support; AL= attachment loss; GC= control group; GL= ligature group; GO= overload group; GLO= ligature and overload group; GLOD= ligature, overload and doxycyline; same letters represent absent of statistical difference.
authors (Klausen 1991; Nociti et al. 2001; Galvão et al. 2003; Gaspersic et al. 2003). Changes in the periodontal tissues have been evaluated by radiographic (Klausen et al. 1989), morphometric (Gaspersic et al. 2003), histometric (Nociti et al. 2001) and molecular biology methods (Karimbux et al. 2001). In all these studies, authors have observed changes in the alveolar bone response and connective attachment loss (Galvão et al. 2003; Lu et al. 1999; Fernandes et al. 2007). It is important to emphasize that the use of one specific method alone is unable to include all the aspects of periodontal disease and, depending on the purpose of the study, the use of combined analysis may be applicable (Klausen et al. 1989). Thus, the present study considered the use of two combined methods that presented complementary aspects. The ligature-induced periodontitis produced radiographic alterations as PBS reduction and histological changes as attachment loss. This combination allowed evaluating of the influence of risk factors for a specific application and regarding therapeutic procedure on the periodontal structures.

The presence of occlusal trauma as a risk factor in periodontal disease has been widely reported in the literature. In a literature review, Harrel affirmed that occlusal overload might produce irreversible alterations when associated with the dental biofilm (Harrel. 2003). These results were confirmed in the present studied, since the characteristics observed in OG were similar to CG, both for PBS or AL evaluation. The radiographic evaluation showed that the mean PBS values for the trauma group were higher than those found for the control group. The interdental area between the first and the second lower molars showed alterations that occurred due to these adaptation and extrusion events. These alterations were noted in alveolar bone crest when ligature was not realized as in OG. Kuhr observed in their study that a period of up to fifteen days post-ligature placement was necessary to observe the tissue alterations that occurred during induced periodontitis and that after this period, the alterations remained stable up to a 60 day observation period (Kuhr et al. 2004). In the present study a 28 day period of induction was enough to evaluate alveolar bone loss in rats subjected to ligature placement. This histological characteristic explains the scenarios (Gaspersic et al. 2003) in which risk factors may aggravate the development of periodontal disease, such as stress or trauma, but are unable to initiate periodontal disease alone. The results of the correlation test confirmed this fact when observation revealed that certain rats that presented lower SOP values did not show high AL values.

Salvi and Lang reviewed the host response modulation involved in reducing tissues alterations that occurred during periodontal disease and discussed the efficacy of LDD therapeutic protocol use to modulate bone loss by means of artificial inhibitors of MMP tissue enzymatic activity (Salvi & Lang, 2004). The period of usage for this medication has been studied in relation to the occurrence of bacterial resistance (Ryan et al. 1996). When the procedure of ligature in association with occlusal overload was compared with groups that only received ligature, the periodontal alterations were aggravated, although the mean values were not statistically different. However, these results showed a tendency for higher values in the groups that received LDD than in the group in which medication was not administered, though these values were statistically similar. The histological evaluation showed that the characteristics observed in rats that received daily doses of LDD presented a reduction in the cement extension without the insertion of periodontal ligament fibrils at molars that received ligature. These alterations were also not statistically significant, thus, using this experimental model, it was not possible to confirm the literature results regarding the risk factor in relation to bone loss modulation and attachment loss in experimentally induced periodontitis in rats associated with occlusal overload. The use of this protocol with a larger sample and the use of molecular biology, a more sensitive method for evaluating the effects of different doses of inflammatory mediators, may be a favorable recourse for verifying these results and a theme for future studies to test the efficacy of this therapeutic protocol.

CONCLUSIONS

Based on these results it was not possible to conclude whether the use of LDD modulated periodontal bone support reduction or attachment loss in induced periodontitis in female rats.

ABSTRACT

Periodontium support degradation is the main biological event in periodontal disease. The aim of this study was to evaluate the influence of systemic use of a low-dose of
doxycycline (LDD) on periodontal bone support (PBS) and attachment loss (AL) in induced periodontitis associated with occlusal overload in rats. This study used 25 female Wistar rats weighing 250g, divided into 5 groups (n=5): CG (control group), LG (ligature group); OG (occlusal overload); LOG (ligature and occlusal overload); LOGD (ligature, occlusal overload and doxycycline). Periodontitis was induced by inserting a ligature around the first mandibular molar (1MDM) for 28 days. In order to obtain occlusal overload at the 1MDM, the occlusal surfaces of the second and third maxillary molars (2MXM and 3MXM) were flattened. 0.14mg of doxycycline was orally administered every day to the LOGD group rats during the experimental period. The rats were sacrificed and the right hemi-mandibles were analyzed by digital radiograph of the PBS(%), whereas the left hemi-mandible was processed for histopathological analysis of AL(mm). The mean values were treated using ANOVA and the Tukey test at 5% level. Results for PBS in the CG (55.49±4.86%) and OG (60.03±3.37%) groups were similar to each other and statistically different from the LG (46.73±4.80%), LOG (51.69±2.84%) and LOGD (57.76±4.50%) groups (p=0.001). For AL, CG (0.11±0.07mm) and OG (0.12±0.10mm) were similar to each other and statistically different from LG (0.41±0.09mm), LOG (0.58±0.12mm) and LOGD (0.54±0.12mm) (p=0.0001). In conclusion, the use of LDD appears not to modulate PBS or AL reduction in rats with periodontitis associated with occlusal overload.

UNITERMS: doxycycline, dental occlusion, periodontics.

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