Evaluation of two morphometric methods of bone loss percentages caused by periodontitis in rats in different locations

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ABSTRACT

Objective: The present study evaluated morphometrically bone loss percentages in experimental periodontitis in rats, comparing different locations (lingual mandible, palatal maxilla and buccal maxilla) and two evaluation methods (distance and area methods). Material and Methods: Ligatures were placed around the maxillary right second molar and around the mandibular right first molar in 14 female Wistar rats. The contralateral molars served as intragroup controls. After 4 weeks, the rats were sacrificed and their mandible and maxilla were removed. The specimens were dissected and stained with methylene blue dye. Bone loss was evaluated by two different methods on the surfaces of the defleshed jaw. In the first method, the distance from the cementoenameal junction (CEJ) to the alveolar bone crest was measured in the roots of teeth associated with ligature. In the second method, the area of bone loss was determined using the alveolar tissue bone, CEJ and the proximal region of roots associated with the ligature as reference. The data were converted to bone loss percentages caused by ligature: (ligated – unligated) x 100/ligated. Results: When comparing the distance and area methods, no statistically significant difference was observed (p>0.05). Both methodologies indicated that the maxilla presented greater bone loss than the mandible and it was more accentuated on the buccal side than on the palatal side (p<0.05). Conclusions: The findings of this study show that both the area and the distance methods can be used to evaluate bone loss caused by ligature placement in rats, and suggest applying the morphometric methodology to the maxilla on the buccal side.

Key words: Experimental studies. Methodology. Periodontal diseases. Rats.

INTRODUCTION

Periodontitis is characterized by the presence of inflammation and progressive destruction of supporting structures of the teeth, including the periodontal ligament, alveolar bone and gingival tissues¹⁴. The use of rat models has been validated in the evaluation of pathogenesis of periodontal diseases¹⁸ and regarding the influence of risk factors, such as nicotine²⁰, alcohol¹³,¹⁵ and diabetes¹³ on disease progression. The induction of periodontal disease in rats can be achieved in many ways. In numerous studies, the use of cotton ligatures is reported, involving the cervical portion of the maxillary second molar¹-²,⁶,⁷,¹⁰,¹⁷,²²,²₃,²₅,²₇ or the mandibular first molar³,⁴,⁹,₁₃,₁₉,₂₀,₂₄. Previous studies have validated the use of morphometric analysis to evaluate alveolar bone loss in ligature-induced periodontitis in rats¹-⁵,⁷,₁₂,₁₄,₁₈,₂₂,₂₃,₂₅,₂₇. According to Klausen¹⁵ (1991), this is the most appropriate methodology to measure periodontal bone loss in dissected rat maxillas. However, these analyses present different locations for evaluation and numerous measurement methods. Alveolar bone loss located on molar buccal
and lingual faces can be quantified by measuring the linear distance of the cementoenamel junction (CEJ) from the alveolar bone crest (ABC) in molar roots. In addition, measurements of the area of bone loss using the alveolar bone tissue, the CEJ and the proximal region of roots as references has also been validated, as has measuring the area of the exposed root. 

The purpose of this study was to evaluate morphometrically the alveolar loss bone caused by experimental induction of periodontitis in rats, comparing different locations (lingual mandible, palatal maxilla and buccal maxilla) and methods (area and distance).

**MATERIAL AND METHODS**

**Animals**

Fourteen 4-month-old adult female Wistar rats weighing 300 g on average were used in the study. All rats were housed under similar conditions and received solid diet (Guabi Nutrilabor, Mogiana Alimentos, Campinas, SP, Brazil) and water ad libitum. The Institutional Animal Research Committee of São José dos Campos Dental School, São Paulo State University, São José dos Campos, SP, Brazil, approved the research protocol.

**Periodontitis induction**

General anesthesia was induced by intramuscular administration with a solution of 13 mg/kg of 2% xylazine hydrochloride (Rompum, Bayer, São Paulo, SP, Brazil) and 33 mg/kg of ketamine hydrochloride (Francotar, Virbac, Roseira, SP, Brazil). To induce periodontitis, cotton ligatures (Coats-Corrente, São Paulo, SP, Brazil) were placed around the cervix of the maxillary second molar and mandibular first molar, both on the right side, leaving the left side molars unligated to serve as controls.

After 4 weeks, the rats were sacrificed by decapitation, the mandibles and maxillas were removed and separated into right and left sides, and the specimens were fixed in 10% formalin solution.

**Morphometric analysis of alveolar loss bone**

The specimens were dissected very carefully to maintain their integrity. The procedure included immersion in sodium hypochlorite for 4 h and mechanical scavenging of the remaining tissue. Next, the specimens were stained with methylene blue dye (1 g/100 mL) for 1 min to demarcate the CEJ.

The specimens were examined under stereomicroscopy. To obtain sufficient reproducibility of the alignment of the image, the buccal cusp tip of the first and second molars should be superimposed on the corresponding lingual/palatal cusp tip. Thus, it is not possible to visualize the occlusal surface. The image was digitalized at 25x magnification for the buccal and palatal sides of the maxilla and for the lingual side of the mandible. Measurements were made on the maxillary second molar and mandibular first molar three times using Image Tool v.3.0 image-analysis software (UTHSCSA, San Antonio, TX, USA), and the mean values were used in statistical analysis. All measurements were made in a blinded fashion to the group to which the rat belonged.

The area method was defined by delimitation of the area of bone loss, using the alveolar bone crest (ABC), the CEJ and the proximal region of roots as reference. The distance method applied by Crawford, Taubman and Smith on digitalized images was used to perform linear measurements from the CEJ to the ABC, on half of each root following the axis. Three measurements were obtained for the mandibular first molar (lingual) and four for the maxillary second molar, two each for the buccal and palatal sides. Both methods can be observed in Figure 1 for the buccal maxilla, in Figure 2 for the palatal maxilla, and in Figure 3 for the lingual mandible.

The bone loss values caused by periodontitis were transformed into bone loss percentages. The percentage of periodontal bone loss (PBL) was measured by two methods (area and distance).
in teeth with (LIG) and without ligature (UNLIG) for the palatal and buccal maxilla, and the lingual mandible. PBL was determined using the following equation: PBL= (LIG – UNLIG) x100/LIG.

Reproducibility
Prior to the statistical analyses, the intraexaminer reproducibility was checked by evaluating two sets of measurements of all specimens with a 1-week interval. Paired t test statistical analysis showed no differences between the mean values obtained for the area method (p=0.4179) and the distance method (p=0.3273). Additionally, Pearson’s correlation coefficient was obtained between the two sets of measurements and revealed a very high correlation in both the area (99%: r=0.99986, p=0.000) and distance methods (99%: r=0.9986, p=0.000).

Statistical analysis
Data were expressed as mean values and standard deviation of percentage (%) of periodontal bone loss (mm or mm²). The t test for independent variables (α=0.05) was used for comparisons between the area and distance methods. One-way ANOVA (α=0.05) and the Tukey’s test for subsequent multiple comparisons (α=0.05)

RESULTS
Morphometric analysis showed no significant differences among PBL values (p>0.05) for the area and distance methods for three locations (buccal maxilla: p=0.6981; palatal maxilla: p=0.0816 and lingual mandible: p=0.3789).
Evaluating the percentage of PBL derived from the cotton ligature insertion in different locations, there was showed significant differences among PBL values (p<0.05). Analysis revealed a greater percentage of PBL in the maxilla, bone loss was more accentuated on the buccal side than the palatal side (p<0.05). On the lingual side of the mandible, there was a lower percentage in PBL values (Table 1).
induction in the maxilla and mandible. This period can range from 15 to 60 days. Several studies in rats used 30 days for periodontitis induction, but Kuhr, et al. (2004) compared the distance morphometric methods to the area method. Moreover, the linear method allows for a comparison of bone loss caused by ligature-induced periodontitis in rats, and suggests applying the morphometric methodology to the maxilla on the buccal side.

REFERENCES

Table 1-Mean and standard deviation of alveolar bone loss (%) at the evaluation location

<table>
<thead>
<tr>
<th>Location</th>
<th>Area method</th>
<th>Distance method</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal maxilla</td>
<td>64.60 (7.13) a**</td>
<td>60.83 (6.94) A***</td>
<td>0.1902</td>
</tr>
<tr>
<td>Palatal maxilla</td>
<td>47.02 (2.54) b</td>
<td>43.11 (4.93) B</td>
<td>0.0638</td>
</tr>
<tr>
<td>Lingual mandible</td>
<td>28.67 (9.13) c</td>
<td>22.74 (8.72) C</td>
<td>0.1643</td>
</tr>
</tbody>
</table>

* independent sample t test (p<0.05). Lower case letters** should be considered in the area method column (ANOVA and Tukey, p<0.05). Means followed by different letters in the distance method column (ANOVA and Tukey, p<0.05). Means followed by different letters differ statistically

DISCUSSION
The most commonly used teeth for periodontitis induction in rats are the maxillary second molar and the mandibular first molar, both of which were evaluated in the present study. Kuhr, et al. (2004) verified that the presence of a cotton ligature caused bone loss, which is detectable by morphometric analysis on day 15 after its placement. Subsequent observation verified the stagnation of bone loss progression up to day 60. Based on that study, it can be concluded that the ligature must remain in place for a minimum of 15 days and the experimental period can range from 15 to 60 days.

Regarding the period of ligature placement, several studies in rats used 30 days for periodontitis induction in the maxilla and mandible. This period was very similar to that used in the present work of four weeks.

Alveolar bone loss in rats can be caused by physiological remodeling or periodontitis induction. The present study identified the effects of ligature on the alveolar bone because the bone loss values obtained in unligated teeth were subtracted from the bone loss values obtained in the presence of ligature. The aim of this methodology was to define the amount of bone loss caused by periodontitis and to determine which area was more sensitive to the bone loss values obtained in unligated teeth. The present study showed that both area and distance measurements can be used to evaluate bone loss caused by ligature-induced periodontitis in rats, and suggests applying the morphometric methodology to the maxilla on the buccal side.