Evaluation of bone loss due to primary occlusal trauma in two experimental models of occlusal overload

Avaliação da reabsorção óssea promovida pelo trauma oclusal primário em dois modelos experimentais de sobrecarga oclusal

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Resumo

Introdução: Trauma oclusal primário (TO) é uma lesão que ocorre no periodonto de inserção de dentes com altura normal devido a forças oclusais que ultrapassam sua capacidade adaptativa. Objetivo: Avaliar histometricamente a reabsorção óssea alveolar na região da furca do 1º molar inferior em ratos submetidos experimentalmente a 2 modelos de sobrecarga oclusal. Material e método: 45 ratos foram randomizados e divididos em 3 grupos: Interferência Oclusal (GI, n = 15) - fixação de um segmento de fio ortodôntico na face oclusal do 1º molar inferior; Sobrecarga Oclusal (GS, n = 15) - desgaste dos cúspides dos molares inferiores contralaterais e dos 2º e 3º molares do lado do 1º molar que teve suas dimensões mantidas; Grupo Controle Negativo (CN, n = 15) - avaliação das dimensões iniciais do ligamento periodontal. Cinco ratos por grupo foram sacrificados aos 14, 21 e 28 dias. Resultado: A avaliação intergrupos mostrou que houve reabsorção óssea significativa em GI (p<0,001) e GS (p<0,01) quando comparados com CN. O grupo GI teve reabsorção óssea significativamente maior quando comparado ao grupo GS em 14 (p<0,01), 21 (p<0,01) e 28 dias (p<0,01). A avaliação intragrupos não mostrou influência significativa do tempo na reabsorção óssea em GI e GS, independentemente da técnica utilizada (p>0,05). A espessura do ligamento periodontal manteve-se estável no grupo CN (p>0,05). Conclusão: GI e GS foram eficazes na reprodução experimental do TO e GI promoveu maior reabsorção óssea alveolar quando comparado a GS, mostrando que o impacto causado pela sobrecarga oclusal no grupo GI aumentou a lesão por TO.

Descritores: Reabsorção óssea; oclusão dental; ferimentos e lesões; ratos.

Abstract

Introduction: Primary occlusal trauma (OT) is an injury of the periodontium with normal height as a result of occlusal forces which exceed their adaptive capacity. Objective: To evaluate, histometrically, the alveolar bone loss in the furcation region of rats experimentally submitted to 2 models of occlusal overload. Material and method: 45 animals randomly divided into 3 groups: Occlusal Interference (OI, n = 15) - fixing an orthodontic wire segment on the oclusal surface of the first lower molar; Occlusal Overload (OO, n = 15) - wearing of the cusps of the lower contralateral molars, the second and third molars next to the first molar that had its dimensions maintained; Negative Control (NC, n = 15) - evaluation of the initial dimensions of the periodontal ligament (PL). Five animals / group were sacrificed after 14, 21 and 28 days. Result: Intergroup evaluation showed significant bone loss in OI (p<0.001) and OO (p<0.01) compared to NC. OI had significantly higher bone loss compared to OO at 14 (p<0.01), 21 (p <0.01) and 28 days (p<0.01). The intragroup evaluation showed no significant influence of time on bone loss in OI and OO, regardless of the technique used (p>0.05). The thickness of the PL remained stable in NC (p>0.05). Conclusion: OI and OO were effective in the experimental reproduction of OT, and OI promoted greater alveolar bone loss compared to OO, showing that the impact of occlusal overload in OI increased the extent of the OT injury. Descriptors: Alveolar bone loss; traumatic dental occlusion; wounds and injuries; rats.
INTRODUCTION

The periodontium comprises root cement, alveolar bone and periodontal ligament itself. The main functions of this sustaining apparatus are to connect the tooth physically, more specifically the root, to the dental socket and mechanically to adapt to the functional demands arising from occlusion\textsuperscript{1,2}. This permits physiological micro-adaptations without rupturing the homeostasis of this system and consequent appearance of injuries.

Ideal functional occlusion is characterized by an appropriate intercuspal tooth, to preserve all physiological components of the stomatognathic system, namely the dental occlusion, the temporomandibular joint, the tooth attachment and the entire neuromuscular mechanism involved in the process of biomechanics of occlusion\textsuperscript{4}. Harmonious occlusion occurs when there is a mandibular relationship in centric and eccentric relation in which there are no interceptive or deflector contacts of the occlusal surfaces\textsuperscript{5}. Changes in the occlusal contacts imply an adaptation of both the periodontal tissues as well as the entire stomatognathic system.

Premature occlusal, defined as any contact between opposite teeth which occurs primarily at the planned closing\textsuperscript{3}; occlusal interference, defined as contact between the teeth which prevents the remaining occlusal surfaces from making stable and harmonious contact, enabling deviation of the mandible from its physiological closing\textsuperscript{6}; and, occlusal overload, defined as a mechanical load that exceeds the adaptive capacity of the support structures\textsuperscript{3,6} and which can occur when the mechanical loads are guided in an eccentric axis can lead the periodontium to collapse. This is because the tissues which compose the support apparatus for the teeth have a limited capacity of adaptation to occlusal forces.

When the functional or parafunctional occlusal forces exceed the capacity for adaptation and repair of the teeth attachment, an injury, limited or progressive, occurs in the supporting tissues. This is called occlusal trauma. When the damage caused by the excessive occlusal forces exceeds the adaptive capacity of the attachment structure of one tooth or a group of teeth with normal height of the periodontium, primary occlusal trauma occurs. Secondary occlusal trauma, in turn, is the injury caused by excessive occlusal forces on a tooth or a group of teeth with reduced height of the periodontium\textsuperscript{1}.

Several studies have been conducted with the aim of understanding the cellular, molecular and genetic mechanisms involved in occlusal trauma\textsuperscript{7-11}. Clinically, however, there is an ethical implication that limits the conduct of prospective studies on occlusal trauma in humans, making it difficult to measure occlusal overload and its implications on bone, cementum and the periodontal ligament\textsuperscript{12,13}. For this reason, experimental studies are frequently conducted using animal models to evaluate the effect of occlusal forces on the periodontium. There are studies of occlusal trauma that have been developed satisfactorily by constructing occlusal apparatus that led to occlusal interference\textsuperscript{4,11} or occlusal overload, experimentally mimicking partially edentulous patients\textsuperscript{15-17}.

The distribution of occlusal forces in the periodontium, due to the type of traumatogenic occlusion, may cause distinct bone injuries according to the force vectors, intensity and frequency of their application. Thus, the aim of the present study was to evaluate, histometrically, the effectiveness of two models of experimental reproduction of occlusal trauma. One model mimicked partial edentulism and the other model by creating occlusal interference and its impact on the occurrence of bone loss in 1\textsuperscript{st} lower molars in rats, subjected to occlusal overload.

MATERIAL AND METHOD

Characteristics of the Sample

Forty-five adult, male Wistar rats weighing between 300 and 400g were used. The animals were housed in plastic cages with access to food and water \textit{ad libitum}. This study was accepted by the Committee for Ethics in Experiments with Animals (Approval # 1245-1).

Induction of Occlusal Trauma and Experimental Design

The 45 animals were randomly divided into 3 groups:

Occlusal Interference Group (OI, N=15): The animals were anesthetized by intramuscular administration of ketamine (1mL/kg) (Dopalen\textsuperscript{8}; Vetbrands LTDA, Jacareí, SP, Brazil) and xylazine hydrochloride (0.3 mL/kg) (Virbaxil\textsuperscript{9}; Virbac do Brasil Indústria e Comércio LTDA, Roseira, SP, Brazil). The maxilla were opened using the Doku apparatus (1966) to insert a segment of orthodontic wire (0.5mm diameter and approximately 1mm length) in the occlusal surface of the first bottom molar randomly chosen by lottery, in order to create occlusal interference, using increments of light-curing resin to attach it (Z100\textsuperscript{9}; 3M, Sumaré, SP, Brazil). The occlusal surface of the selected molar was previously cleaned using a microbrush, followed by conditioning of the occlusal surface with 37% phosphoric acid (Villevie\textsuperscript{8} Dentalville do Brazil, Joinville, SC, Brazil), and adhesive was applied (Single Bond\textsuperscript{9}; 3M, Sumaré, SP, Brazil) according to manufacturer’s specifications. The diameter of the orthodontic wire standardized the height of the occlusal interference, with the resin inserted up to the limit of the height of the wire and not going beyond it. The animals were euthanized after 14, 21 and 28 days..

Occlusal overload group (OO, N=15): The animals were anesthetized and had their maxilla opened, as described above. Occlusal wear was performed on the occlusal surface of all cuspids of the lower 2\textsuperscript{nd} and 3\textsuperscript{rd} molars next to the 1\textsuperscript{st} molar that had its vertical dimensions kept and of the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} contralateral molars. Thus, a n° 1016HL diamond drill-bit (KG Sorensen, Cotia, SP, Brasil), attached to a cooled, high-speed motor (MRS 400 PB, Dabiatante, Ribeirão Preto, SP, Brasil), was used to create experimentally a situation of occlusal overload. The animals were euthanized after 14, 21 and 28 days.

Negative Control Group (NC, N=15): The animals were anesthetized but did not receive treatment. This group was included to obtain values related to the dimensions of the periodontal ligament (PL) at the baseline, and to confirm the efficacy of the induction models of occlusal trauma. The animals were euthanized after 14, 21 and 28 days.
Euthanasia of the Animals and Histological Processing

The animals underwent general anesthesia, as described in the period of OT induction, and euthanized using transcardiac perfusion with 10% formol in 0.1M phosphate buffer (pH 7.0) for approximately 10 minutes for each animal. This technique was selected to provide better setting of the pieces and, consequently, a better result of the histological processing for histometric purposes. Following the setting, the mandible was removed and hemi-sectioned at the symmetry. The material obtained was immersed in 10% formol with phosphate buffer (pH 7.0) for 24 hours. The hemi-mandibles were decalcified using 10% EDTA for 16 weeks at room temperature; the solution was renewed daily. After demineralization, the specimens were dehydrated in increasing concentrations of ethanol, diaphanized in xylene and embedded in paraffin. Longitudinal, mesio-distal sections of 6μm thickness were obtained using a microtome (Leica RM2155, Germany). The slices were stained using hematoxylin and eosin.

Histometric Analysis

Interradicular bone loss

Serial slices of 6μm thickness were obtained from the vestibular bone plate, and the slices in which the bifurcated region was identified were selected. To estimate the volume, the first slice was discarded and the remaining equidistant slices were selected according to the total number of histological slices obtained for each tooth. Thus, 10 equidistant, histological slices were selected for each tooth for histometric evaluation, and scanned at 50X magnification (5X objective and 10X ocular, Axioskop 2 plus, Zeiss, Jena, Germany). The Computer Eye (Digital Vision, Dedham, MD, USA) program was used to capture the images of the cuts. Using the point system of a checkerboard reticulum, with the aid of the Image-Pro® (Media Cybernetics, Silver Spring, MD, USA) software, the area of conjunctive tissue coming from the loss of bone tissue from the interradicular region, or the thickness of the periodontal ligament of the CN group, was measured. The reticulum comprised squares of 0.08mm edges and 0.0064mm² areas. The reticulum was positioned such that it always included coronary and radicular dentin and bone tissue. The points that coincided with the intersections of the edges of the adjacent squares, inside the area of conjunctive tissue present in the interradicular region as a consequence of bone loss, were computed. The area of bone loss in the furcation region was determined from the average of the readings of the 10 equidistant slices for each tooth, expressed in mm².

Calibration of the examiner

To evaluate the calibration of the examiner, an intra-class correlation was conducted prior to the final readings. Twenty slices were selected randomly, and their measurements were made and repeated 3 weeks after the first measurement. The high value of the correlation coefficient (r=0.989; Confidence Interval 95%; 0.972-0.996) shows consistency in the reproducibility of the reading, showing good calibration of the examiner.

RESULT

Normality of the sample was demonstrated by the Shapiro-Wilk test. Thus, the ANOVA test (α = 0.05) with the Tukey test as the post-test were used for the detection of differences (α = 0.05).

Intragroup analyses of the OI and OO groups showed no statistically significant differences (p>0.05). This demonstrates that the bone loss observed at 14 (Figures 1A and 1D) was similar to that observed at 21 (Figures 1B and 1E) and 28 (Figures 1C and 1F) days for both groups. That is, there was no influence of time on the increase of bone loss coming from OT after 14 days of experiment for the OI and OO groups (Table 1). The NC group (Figures 1G, 1H and 1I) showed no change in the dimensions of the PL at 14, 21 and 28 days (p>0.05), demonstrating that time did not influence the initial dimensions of the PL.

Intergroup analysis showed significant difference (p<0.05) when comparing groups OI x OO at 14 (0.67 ± 0.13 x 0.51 ± 0.19) (Figures 1A and 1D), 21 (0.66 ± 0.12 x 0.56 ± 0.14) (Figures 1B and 1E) and 28 days (0.74 ± 0.06 x 0.584 ± 0.21) (Figures 1C and 1F). This shows greater bone loss in Group OI for all periods of evaluation. When comparing groups OI x NC at 14 days (0.67 ± 0.13 x 0.32 ± 0.04) (Figures 1A and 1G), 21 days (0.66 ± 0.12 x 0.28 ± 0.03) (Figures 1B and 1H) and 28 days (0.74 ± 0.06 x 0.22 ± 0.02) (1C and 1I) and OO x NC at 14 days (0.51 ± 0.19 x 0.32 ± 0.04) (Figures 1D and 1G), 21 days (0.56 ± 0.14 x 0.28 ± 0.03) (Figures 1E and 1H) and 28 days (0.584 ± 0.21 x 0.22 ± 0.02) (Figures 1F and 1I), significant differences were observed, showing greater bone loss for OI and OO compared with the NC group in all periods (p<0.01). This shows that both proposed experimental models were effective in reproducing primary occlusal trauma. Results are shown in Table 1.

DISCUSSION

It is known that there is alveolar bone loss in occlusal trauma1,8,15, because all periodontal support tissues are involved. However, there is no information in the literature about the type of occlusal condition and its pathogenic intensity in the periodontium. Due to

| Table 1. Mean ± Standard Deviation of bone loss (mm²) in the furcation region of the 1st lower molars, based on the experimental model and on time |
|----------------|----------------|----------------|
| Periods        | Treatment      |              |
|                | OI             | OO            | NC            |
| 14 days        | 0.67 ± 0.13A   | 0.51 ± 0.19B  | 0.32 ± 0.04 C |
| 21 days        | 0.66 ± 0.12A   | 0.56 ± 0.14B  | 0.28 ± 0.03 C |
| 28 days        | 0.74 ± 0.06A   | 0.584 ± 0.21B | 0.22 ± 0.02 C |
| P (linear)     | 0.4464 NS      | 0.4493 NS     | 0.421 NS      |

Mean ± SD of detectable alveolar bone loss area on furcation region of first lower molars (mm²) based on treatment and time. Means followed by different letters in the raw differ by ANOVA followed by Tukey’s test (p<0.05). NS= not significant. OI= occlusal interference; OO= occlusal overload; NC= negative control (without treatment).
ethical limitations on prospective evaluation of OT in humans, 2 experimental models in rats were developed for the present study, with the goal of evaluating the consequence of the type of occlusion on the development of bone loss in primary OT. In one of the models, occlusal interference[7,8,11] was created to imitate experimentally the occlusal interferences and premature occlusal contacts that lead to the increase in the vertical dimension of occlusion, leading to occlusal instability by altering the force vectors and changing the magnitude of the applied forces to one tooth. In the other model, the unilateral vertical dimension was reduced, creating occlusion on only one of the first molars, with the goal of reproducing experimentally the cases of partial edentulism[15], also creating occlusal overload experimentally.

Intragroup analysis showed that there is no significant difference in the extent of bone loss verified in the alveolar bone in the interradicular region of the first lower molar of the animals at 14, 21 and 28 days (p>0.05). These data lead to the belief that the periodontium underwent functional adaptation, having adapted to a pathological state due to significant changes in the magnitude, frequency of event and change in direction of the force vectors of the occlusal demand[18,19]. Thus, it may be hypothesized that the 1st lower molar of the animals may have undergone functional adaptation due to possible tooth migration or inclination involving force vectors[20], permitting control of the damage during the applied experimental period (14, 21 and 28 days). Consequently, no histometrically significant intragroup differences were seen regarding bone loss in OI and OO during the evaluation periods of primary OT.

Intergroup analysis showed the effectiveness of both models in the development of OT, showing significant bone loss in the furcated region of the 1st lower molar when comparing the OI and OO groups with the NC group (p<0.01). The OI x OO intergroup analysis showed that the OI group lost significantly more bone when compared to the OO group at days 14, 21 and 28 (p<0.05). This shows that the occlusal interference model seems to be more harmful to the alveolar bone when compared to the overload model applied to OO. This may have occurred due to the intensity and

Figure 1. Photomicrographs illustrating periodontal area and alveolar bone loss on 1st molars furcation region in 50 x magnification (5 x objective and 10 x ocular), during experimental periods 14, 21 and 28 days. OI= occlusal interference (A, B, C); OO= occlusal overload (D, E, F); NC= negative control (G, H, I); d= dentine; CT= connective tissue from alveolar bone loss; AB= interradicular alveolar bone; PL= periodontal ligament; Bar= 200μm.
direction of the forces in the furcation region of these teeth and the consequent proportional activation of chemical mediators facing the traumatological demand. It also may be hypothesized that there was greater activation of osteoclasts by the over-regulation of the binding of the receptor of the activator of the nuclear factor Kappa B (RANK/RANKL) and/or by the reduction in the production of osteoprotegerin (OPG). This would allow greater differentiation of the pre-osteoclasts in osteoclasts, resulting in greater bone loss in the OI group. There may likely have occurred greater magnitude of the forces, greater frequency of the occlusal contact events and alteration in the direction of the force vectors on the periodontium of the rats.

The scientific literature that uses animal models for experimental reproduction of occlusal trauma are limited in the comparisons of the results of the present study with previous studies, since most of them use the secondary or the combined occlusal trauma model. The data should be evaluated with the constraints that the animal model provides, because none of the existing animal models duplicate human physiology. However, they are of great value when a prospective clinical evaluation of the development of an injury is impossible. More histological, histochemical and immunological studies should be conducted, using both shorter and longer times, for greater clarification of the damage caused by OT to the alveolar bone and to the other tissues of the periodontium.

Thus, within the limits of the present study, it may be concluded that the models of occlusal interference (OI) and occlusal overload (OO) effectively reproduced primary OT. It also may be seen that there was greater alveolar bone loss in the group in which occlusal interference was used.

REFERENCES


CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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