The effect of traumatic dental occlusion on the degradation of periodontal bone in rats

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ABSTRACT

Context: A better understanding of the relation between traumatic dental occlusion and periodontal changes is needed.

Aims: This study aimed to evaluate the pattern of osteoclastic activity in the periodontal bone in front of the traumatic dental occlusion in rat molars.

Patients and Methods: Traumatic dental occlusion (TO) was induced in twenty rats, which were sacrificed after periods of 2, 5, 7, and 14 days. Transversal histological sections of both jaws were stained with tartrate-resistant acid phosphatase and hematoxylin and eosin. Mann–Whitney U-test was used for group comparison, and Pearson's correlation test was applied for the number of osteoclasts and bone area (BA).

Results: Traumatic dental occlusion caused an increase in the number of osteoclasts in the bone of the upper and lower right first molar from days 2–5 to 2–14, respectively. In the TO groups, the number of osteoclasts on the lamina dura and in the center of the alveolar bone septum, respectively, increased almost 4-fold and 9-fold in the lower jaw; and 3-fold and 5-fold in the upper jaw, during all periods. In the TO groups, the BA of the alveolar bone septum was substantially reduced. There was a negative correlation between the number of osteoclasts and BA in both jaws during all experimental periods.

Conclusions: Traumatic dental occlusion increases osteoclast activity in the alveolar lamina dura and in the center of alveolar bone and stimulates a higher degradation in the center of the alveolar bone septum.

Key words: Alveolar bone, dental occlusion, osteoclasts, periodontium, traumatic tissues, whereas the absence or excess of occlusal load results in disharmonic functioning of periodontal tissue.[7,8]

With respect to occlusal force, the mechanism for transmission and neutralization of occlusal forces consists of several elements that will prevent excessive occlusal pressure, including periodontal ligament fibers, tissue fluid in the periodontal space, proprioception of the periodontal ligament, amorphous ground substance, vessels, trabecular bone architecture, and tooth root shape.[9]

The relation between traumatic dental occlusion and periodontal changes needs to be better understood to create approaches that suit individual cases. Discussion centers around the question if traumatic dental occlusion is a co-destructive factor of periodontal disease,[1-3] and how it can affect the periodontium, considering that traumatic dental occlusion does not alter clinical probing depth.[4-6]

Normal occlusal function is a mechanical stimulus necessary to maintain homeostasis of the periodontal

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Traumatic dental occlusion is often caused by an occlusal interference, such as high restorations or prostheses, and/or parafunctional habits (e.g., clenching, bruxism, biting on nails, ice, pens and lips). It can cause a variety of destructive biological effects on pulp tissue, the periodontium, alveolar bone, masticatory muscles, and temporomandibular joint.

In primary or secondary occlusal trauma, the ultimate goal of successful management of mobile teeth is to restore function and comfort by establishing stable occlusion, which promotes tooth retention and periodontal health.

In cases of traumatic dental occlusion, the signs that can be most commonly observed through radiological analyses are root resorption and fractures, vertical reduction of the interdental septum, condensation and radiolucency of the alveolar bone, increased width of the periodontal ligament space and angular bone defects.

However, even though the occlusal trauma concept is widely accepted, the influence of traumatic dental occlusion on osteogenesis and osteoclastogenesis in the alveolar bone remains controversial. The factors that contribute to condensation and radiolucency of the alveolar bone are not known. The most recent papers that reviewed literature about the relationship between traumatic dental occlusion and periodontal disease point to the need for more studies to explain this association.

Normal bone structure is determined by a balance between bone formation (by osteoblasts) and bone resorption (by osteoclasts) orchestrated by osteocytes, the bone cells embedded in bone that perceive mechanical loading of the bones. The general concept is that bone structure is in part determined by mechanical loading: Too low loading (disuse) activates or recruits osteoclasts to degrade bone and normal loading (functional use by mastication) stimulates osteoblasts to produce bone. The hypothesis is that traumatic dental occlusion disrupts the normal balance between bone formation and bone resorption in the bone surrounding the teeth by mechanical overloading which will damage the bone structures; this recruits osteoclasts to remove the damaged structures. This study aimed to evaluate the pattern of osteoclastic activity in the periodontal bone in front of the traumatic dental occlusion in rat molars, by analysis of osteoclast’s number in the lamina dura and in the center of the alveolar bone septum; and the alveolar bone area (BA).

PATIENTS AND METHODS

All experimental procedures were carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and following approval by the Animal Care Committee of the Dentistry School of Araçatuba (UNESP) agree with ethical principles in animal research (COBEA) and was approved by CEUA (2012-00980), forty Wistar rats (Rattus norvegicus albinus) aged 7 weeks were selected. They were kept in cages with five animals each and given granulated food and water ad libitum. The environment was kept at a constant temperature of 22°C (±2°C) and 50% (±10%) humidity, and light/dark cycles of 12/12 h.

Prior to the experimental section, the animals were intramuscularly anesthetized with a solution of ketamine hydrochloride (25 mg/kg, Vetanarcol, Laboratorios König, Argentina) and xylazine (10 mg/kg Coopazine, Coopers, Brazil).

To evaluate the influence of mechanical loading on the alveolar bone after traumatic dental occlusion, the animals were divided into two groups: A control group and a traumatic dental occlusion group. The control group (n = 20) consisted of rats of the same age as those in the experimental group. To enable comparison under normal conditions, these animals were not submitted to any experimental condition. In the traumatic dental occlusion group (n = 20), excessive mechanical loads were induced by increasing the height of the lower right first molars by direct filling using 37% phosphoric acid etchant for enamel and dentin (FGM, Brazil), microbrushes (Microbrush International, Grafton, USA), scotchbond multi-purpose light adhesive (3M ESPE, Saint Paul, USA), Estelite Σ-Quick composite resin (Tokuyama Dental Corp, Japan), and photopolymerizer (Dabi Atlante, Ribeirão Preto, Brazil), following manufacturer’s instructions. A flat high occlusal table was created at the highest occlusal cusp. A piece of ligature wire 0.20 mm (0.008”) (Morelli, Sorocaba, Brazil) was attached to the surface of the composite filling. Prior to the restoration, microretentions were made with Carbide burs FG ¼ (Beavers Dental, Canada) at high speed, using a handpiece with water. The restorations were performed by a single trained dentist to maximize standardization of the results.

The study periods were 2, 5, 7, and 14 days, for both groups. Animals were excluded in case they died of natural causes, or if they lost the occlusal composite filling. Twelve rats were lost, 5 in the control group and 7 in the traumatic dental occlusion group. None of the group per period had less than three samples for the statistical analyses.

After anesthesia, transcardial perfusion was performed. An intraventricular injection of heparin (0.1 ml/5000 UI/ml) was administered. After 1 min, 100 ml saline was perfused through the aorta, followed by a mixed solution of 300 ml paraformaldehyde fixation at 4% (Sigma Chemical Co., St. Louis, MO, USA) and 200 ml phosphate-buffered saline (PBS) at 0.1 M, pH 7.4. 4°C (Sigma Chemical Co., St. Louis, MO, USA).
After dissection, the specimens were washed in PBS and kept in 4% paraformaldehyde (Sigma Chemical Co., St. Louis, MO, USA) for 24 h before decalcification with ethylene diamine tetra acetic acid disodium at 10% for 20 days. The specimens were processed with progressive dehydration in ethyl alcohol, cleared with xylene, impregnated with paraffin at a low fusion temperature (56–58°C) for 3 h and embedded according to standard protocols.

Transversal histological sections 5 µm thick of the right upper and lower first molar and surrounding tissues were obtained with an automated microtome (Leica SMR 2000), transferred to a bain-marie (40–50°C) and then collected using silanized slides.

Some sections were stained with hematoxylin and eosin (HE) using conventional methods, while others were submitted to tartrate resistant acid phosphatase (TRAcP) staining.

To show TRAcP activity, sections were deparaffinized, rehydrated, and rinsed in PBS. For reactivation 0.1 M Tris pH 9.0 solution was used, overnight at room temperature. Next, the sections were incubated with a solution containing 30 mg Naphtol-AS-BI phosphate (Sigma D4254, Sigma Aldrich Chemie GmbH, Taufkirchen, Germany), 0.5 ml Dimethylformamide (Sigma D4254, Sigma Aldrich Chemie GmbH, Taufkirchen, Germany), 9 ml acetate buffer in PV, 0.1 ml MgCl₂, 0.1 ml KNa-tartrate 4H₂O, 0.3 ml pararosaniline and 0.3 ml NaNO₂; for 1 h at 37°C. Subsequently, the sections were rinsed in PBS and counterstained with Mayer hematoxylin.

Of each specimen, a number of three sections with a distance of 40 µm in between were selected to perform histomorphological analysis and group comparison at the selected intervals throughout the experimental period. The sections were observed using an Aristoplan light microscope (Leica-Aristoplan, Solms, Germany) and micrographed with an AxioCam MRc digital camera (Carl Zeiss, Oberkochen, Germany). Visual fields of the upper and lower right first molar and surrounding tissues of each animal were collected using ×20 objectives and analyzed with the program Axionvision Rel 4.0 (Carl Zeiss, Oberkochen, Germany). For quantitative analysis, the images were processed using ImageJ.

The sections stained with HE were used to conduct qualitative and quantitative analyses of the alveolar BA. An area of 400 µm², magnified 100 times, at the interradicular alveolar bone between the roots of the right upper and lower first molar was used. The BAs were quantified as a percentage of the total areas, it includes mineralized bone and osteoid. The number of osteoclasts (TRAcP positive cells) was counted on the lamina dura and in the center of the alveolar septum (600 µm × 800 µm) of the first lower and upper molar at the right side [Figure 1].

DISTANCE was made between mononuclear and multinuclear TRAcP cells.

To avoid bias during analysis, examiners were not informed to which group the images belonged beforehand.

The data were analyzed using IBM SPSS 20.0 (IBM, Armonk, NY, USA) at α = 0.05. Mann–Whitney’s U-test was used for group comparison, and the Pearson’s correlation test was applied for number of osteoclasts and BA. Data are expressed as mean ± standard deviation.

RESULTS

Representative histological observations are shown in Figures 1-3. These analyses were limited to the cervical region of the alveolar bone of the right-side first molars of the maxilla and mandible.

The weight gain, plaque index, and gingival inflammation of the animals in this study showed no clinical difference between the control and experimental group.

The control group showed a normal pattern of histological characteristics during the whole experimental period. The alveolar bone showed basophilic concentric lines, revealing an incremental pattern of bone matrix apposition.
In both groups, the distal side of the roots contained significantly more osteoclasts than the mesial side during the whole experimental period [Figure 1c, d, g and h].

In the alveolar bone septum of both upper and lower jaws of the TO group, the BA per unit of tissue was lower than in control jaws [Figures 1b, f, 2f, h and 3f, h]. In these areas, osteoclasts were present on the lamina dura and in the medullary spaces of the alveolar bone [Figures 1c, d, g, h, 2a-d and 3a-d]. On day five, alveolar bone resorption continued, mainly in the center of alveolar septum [Figures 1d, 2d and 3d]. On days 7 and 14 [Figures 1f, 2h and 3h], moderate remodeling of alveolar bone still occurred, with decreased BA and bone resorption, though less than previous groups.

In control groups, the total number of TRAcP positive cells was about 10-fold higher in alveolar bone surface facing the roots than in the center of alveolar bone septum with substantially more (2–3-fold) mononuclear than multinuclear cells [Tables 1 and 2]. In the TO group, the total number of TRAcP positive cells was about 2.5–4 times higher than in control group and again this population contained 2–3 times more mononuclear than multinuclear cells. TO also increased the number of TRAcP-positive cells in the center of alveolar septum by at least 10-fold though their absolute numbers in controls (2–6 cells) were much lower than on the lamina dura (21–35 cells).

On the lamina dura of upper molars, the increase in TRAcP positive cells happened the first 5 days, with a trend to sustain elevated levels of TRAcP-positive cells at days 7 and 14 days though these values were not statistically significant. In the lower jaw of the TO groups, more osteoclasts were present in the first 7 days in TO group with similar differences in the numbers of TRAcP-positive cells between surface and center of septum as found in upper jaw. Also, on the lamina dura of the lower jaws, there were 3–4-fold more TRAcP-positive mononuclear cells than in the center of septum [Tables 1 and 2].

Morphometry indicated less BA in the TO group in the center of alveolar bone septum in the lower jaw than in the upper jaw [Figures 4 and 5]. For most periods examined, the number of TRAcP-positive cells in the center of bone septum in the TO groups was significantly negative correlated with BA in both the maxilla and mandible [Tables 3 and 4].
Table 1: Number of osteoclast in the alveolar bone in the right first molar following traumatic dental occlusion

| Right first upper molar | Days of the Experiment | Mean±SD          | p     |
|-------------------------|------------------------|------------------|-------|
|                         | Control                | Traumatic dental occlusion |       |
| Mononuclear osteoclasts on the lamina dura | 2  | 19.6±8.1       | 57.2±13.0 | 0.02* |
|                         | 5  | 14.3±6.3       | 56.5±25.4 | 0.05* |
|                         | 7  | 13.6±6.1       | 47.5±6.3  | 0.20  |
|                         | 14 | 21.0±1.8       | 50.1±10.6 | 0.20  |
| Multinuclear osteoclasts on the lamina dura | 2  | 10.5±4.3       | 26.2±7.2  | 0.02* |
|                         | 5  | 8.5±5.3        | 36.5±19.8 | 0.05* |
|                         | 7  | 8.0±5.6        | 31.0±7.0  | 0.20  |
|                         | 14 | 14.3±4.2       | 39.1±4.4  | 0.20  |
| Osteoclasts total on the lamina dura | 2  | 30.2±12.3      | 83.4±20.1 | 0.02* |
|                         | 5  | 22.6±11.5      | 93.0±45.2 | 0.05* |
|                         | 7  | 21.6±11.7      | 78.5±13.3 | 0.20  |
|                         | 14 | 35.3±5.3       | 89.3±15.0 | 0.20  |

SD=Standard deviation

Table 2: Number of osteoclast in the alveolar bone in the lower right first molar following traumatic dental occlusion

| Right first lower molar | Days of the experiment | Mean±SD          | p     |
|-------------------------|------------------------|------------------|-------|
|                         | Control                | Traumatic dental occlusion |       |
| Mononuclear osteoclasts on the lamina dura | 2  | 15.8±6.7       | 73.2±6.9  | 0.02* |
|                         | 5  | 15.0±2.1       | 81.7±29.3 | 0.05* |
|                         | 7  | 17.5±4.2       | 56.3±15.1 | 0.01* |
|                         | 14 | 18.9±18.8      | 67.8±18.8 | 0.05* |
| Multinuclear osteoclasts on the lamina dura | 2  | 8.3±4.3        | 25.5±6.2  | 0.02* |
|                         | 5  | 11.5±1.6       | 27.7±11.0 | 0.05* |
|                         | 7  | 14.7±2.3       | 20.3±5.1  | 0.11  |
|                         | 14 | 8.1±9.4        | 27.9±8.7  | 0.05* |
| Osteoclasts total on the lamina dura | 2  | 24.1±10.9      | 98.7±11.8 | 0.02* |
|                         | 5  | 26.6±3.4       | 109.3±39.8 | 0.05* |
|                         | 7  | 32.2±6.3       | 76.5±19.4 | 0.01* |
|                         | 14 | 27.0±28.2      | 95.7±25.3 | 0.05* |
| Mononuclear osteoclasts in the center of alveolar bone septum | 2  | 2.4±0.8        | 15.8±4.5  | 0.02* |
|                         | 5  | 2.6±1.7        | 26.7±21.8 | 0.05* |
|                         | 7  | 3.0±1.3        | 24.0±6.9  | 0.01* |
|                         | 14 | 2.5±0.7        | 16.5±4.2  | 0.05* |
| Multinucleated osteoclasts in the center of alveolar bone septum | 2  | 0.3±0.3        | 9.0±3.9   | 0.02* |
|                         | 5  | 0.5±0.9        | 10.2±12.1 | 0.11  |
|                         | 7  | 0.9±0.6        | 9.0±4.5   | 0.01* |
|                         | 14 | 0.4±0.7        | 5.7±1.8   | 0.05* |
| Osteoclasts total in the center of alveolar bone septum | 2  | 2.7±0.9        | 24.8±7.5  | 0.02* |
|                         | 5  | 3.2±1.9        | 36.9±33.8 | 0.05* |
|                         | 7  | 3.9±1.9        | 33.0±10.6 | 0.01* |
|                         | 14 | 3.0±1.4        | 22.5±5.0  | 0.05* |

p values are for comparison between the two groups. Mann-Whitney’s U-test was used for continuous variables. Data are presented as the mean±SD. *p≤0.05.

DISCUSSION

Many experimental studies in animals have been performed to examine the influence of occlusion status on tooth-supporting tissues. However, at present, few quantitative analyses of occlusal trauma in bone tissue exist. In some studies, experimental traumatic dental occlusion was simulated by raising the vertical dimension of occlusion.\cite{21-24} The destruction of alveolar bone through traumatic dental occlusion has been assessed using radiography,\cite{6} tomography,\cite{25} histological analysis,\cite{3,7,25-31} and polymerase chain reaction,\cite{16,32,33} and the effect of
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In the first 7 days following traumatic dental occlusion, a pressure increase in the interstitial fluid of the periodontal ligament was observed after 48 h. Until the 5th day, the width of the periodontal ligament space decreased, to return to normality on day 7–120, likely as a result of bone remodeling. The reduction of mechanical force in the periodontium by wearing of the filling and by recovery of the periodontal ligament width most likely reduce osteoclast activity in the alveolar bone. These results show that until day 14, osteoclastic activity was still higher in the TO group, probably because there was not yet a new balance between mechanical pressure and adaptation of the periodontal tissue in the periodontal ligament width presented.

Table 3: Nonparametric correlation between the number of osteoclasts and bone area in the center of the alveolar septum in the upper jaw, presented by rank correlation

| Maxilla | Number of osteoclasts in the center of alveolar bone septum x bone area |
|---------|---------------------------------------------------------------|
| Day 2   | −0.833                                                      |
| Day 5   | −0.750                                                      |
| Day 7   | −0.800                                                      |
| Day 14  | −0.900                                                      |
| 0.01**  | 0.05*                                                        |

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level.

Table 4: Nonparametric correlation between the number of osteoclasts and bone area in the center of alveolar septum in the lower jaw, presented by rank correlation coefficient

| Mandible | Number of osteoclasts in the center of alveolar bone septum x bone area |
|----------|------------------------------------------------------------------------|
| Day 2    | −0.905                                                                 |
| Day 5    | −0.775                                                                 |
| Day 7    | −0.750                                                                 |
| Day 14   | −0.786                                                                 |
| 0.00**   | 0.04*                                                                  |

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level.

These results show differences in the BA between the maxilla and mandibles, presenting quicker bone degradation in the maxilla. It can be explained by the fact that the spongious bone has a higher turnover; and responds quicker to mechanical, chemical, and hormonal stimuli.

The age of the animals may also influence the changes reported here. Young rats, aged 7 weeks, were used in the study. Osteoclasts were seen at the distal side of the roots in both groups. In the animals subjected to frontal traumatic dental occlusion, number of the osteoclasts increased, while the distal side of the root continued to show high concentrations of osteoclasts as well. The continuous growth of rat jaws and their lengthening in posterior direction may be responsible for the distal movement of the teeth although the rate of movement rapidly decreased with age.

The control group is an important measure needed to accurately evaluate the effect of the experiment. Considering that occlusal interference on one side can also alter normal occlusion on the other side in one animal, it is necessary for the control group to consist of untreated animals. The use of opposite sides as a contradistinctive or control group to reduce the difference between individual animals is not recommended; this goal can be better achieved by increasing the number of animals.

Although the number of osteoclasts is higher in the bone around the root, the resorption of the alveolar septum takes place at a higher intensity in the center of the septum, maybe because in both C and TO groups mononuclear TRAcP-positive cells were 2–3 times more abundant than multinuclear cells in the surface. In transversal slides, it can be observed that the resorption process on the lamina dura of the alveolar septum has initiated; however, the height reduction did not correspond with the turnover percentage at the center of the septum. This may indicate that difficulty for flow of fluids and bone demineralization that accompanies or follows osteoclast activity probably take part in the bone degradation process.
degradation in front of traumatic dental occlusion with more intensity at the center of alveolar septum.[37,38]

CONCLUSION

Traumatic dental occlusion of teeth increases the recruitment of mononuclear and multinuclear osteoclasts in the lamina dura and in the center of the alveolar septum, and stimulates a higher degradation of alveolar bone at the center of the septum. This increase in the number of osteoclast is 3 to 4 times greater at the center of the alveolar septum compared to the number of osteoclasts in the lamina dura.

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Conflicts of interest

There are no conflicts of interest.

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