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Technical Note

Experimental Model of Occlusal Trauma in Mouse Periodontal Tissues

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Abstract: The aim of this study was to establish a model, which can be used to investigate the response of periodontal tissues to excessive occlusal loading in mice by observing histopathological changes. The experiment was performed on ten 7-week-old ddY male mice. Under general anesthesia by intraperitoneal injection pentobarbital sodium, a micro-plus-screwpin (head part, 1.7mm in diameter, thickness 0.5mm thickness) was screwed into the pulp cavity of the upper-left-first molar. R_mCT images of the experimental site indicated the occlusal contact position between the upper- and lower-left-first molars during the experimental periods. A micro-plus-screwpin was maintained at a constant position on the occlusal surface throughout the experimental period. Histopathological changes of the periodontal ligament at the furcation lesion of the lower-left-first molar and its surrounding periodontal tissues were observed under a light microscope. The densities of deeply dyeing cells in hematoxylin staining with a round-shaped nucleus were increased in the periodontal ligament, with a peak effect of day 4. Multinucleated giant cells appeared in the central lesion of the periodontal ligament on day 7. The distribution of resorption on the surface of both of the cementum and the alveolar bone was accompanied by multinucleated giant cells, which expanded rapidly from day 7 to day 14. These results showed that histopathological changes of periodontal tissues to excessive occlusal load were observed at the furcation area of molar teeth. The present method confirmed the effectiveness of the experimental model to examine the occlusal trauma on periodontal tissues produced by excessive occlusal load.

Key words: Occlusal trauma, Periodontal tissue, Experimental model, Mouse, Histopathology

Introduction

Occlusal trauma is defined as an injury to the attachment apparatus of a tooth as a result of excessive occlusal forces[1]. It has been proved in many studies that occlusal trauma can cause a variety of destructive biological effects on periodontal tissues[2-5]. A number of animal experiments have been performed in attempts to evaluate the influence of occlusal trauma on the initiation of periodontal disease. Trauma from occlusion in the presence of periodontal tissues along with plaque-induced inflammation may have an important contributory role in the progression of periodontal disease[6-9]. Occlusal forces may alter the path of spread of gingival inflammation and induce angular bone destruction accompanied by infrabony pocket formation[10,11]. It is thought that the force-pressure from excessive occlusal loading is centered at periodontal tissues in the furcation area of a molar tooth. As the forces that cause occlusal trauma are usually intermittent, it is important to observe the tissue responses under such conditions[12]. However, the precise mechanism of periodontal tissue breakdown under the influence of the excessive occlusal loading remains unclear, because of the lack of a convenient experimental model *in vivo*.

The purpose of this study was to establish a model in mice which can be used to investigate the response of periodontal tissues to excessive occlusal loading by observing the histological changes.

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Materials and Methods

Experimental model for excessive occlusal loading

Ten 7-week-old ddY male mice were examined in the present study. Each mouse was placed on the working-table in a dorsal position under general anesthesia by intraperitoneal injection of pentobarbital sodium (40 mg/kg). Using a #1/4 jet carbide bar (#432296 1/4, Shofu Inc., Kyoto, Japan) and a straight hand-piece drill, a guiding hole was created in the occlusal surface of upper-left-first molar. A micro-plus-screwpin (head part: 1.7 mm in diameter and 0.5 mm thickness, Ohsato, Saitama, Japan) was

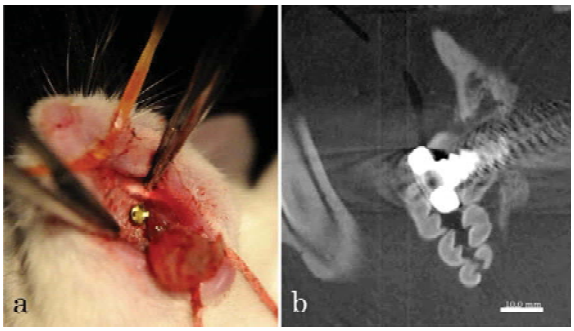

Figure 1. Experimental procedures. a: The occlusal surface of the upper left-first molar was raised by 0.5mm at the head of the micro-plus-screw pin. b: R_mCT image of the experimental site indicated the occlusal contact position between upper-left-first molar and lower left -first molar during the experimental period.

screwed into the guiding hole and fixed in the pulp cavity. The occlusal surface of the upper-left-first molar was raised by the 0.5 mm thickness of the head of the micro-plus-screwpin (Fig. 1a).

R_mCT was used to confirm the occlusal contact between upper-left-first molar and lower-left-first molar.

Sample preparation and histopathological examination

At 4, 7 and 14 days after increasing occlusal height, the mice were sacrificed by an overdose of pentobarbital sodium. Three mice served as a control group. Specimens containing the furcation area of the lower-left-first molar were fixed in 10 % neutral buffered formalin solution, demineralized in 10 % EDTA, dehydrated in increasing series of alcohol in a routine manner and embedded in paraffin. Bucco-lingual serial sections of 4 μm thickness were prepared and stained in hematoxylin-eosin.

Histopathological changes of the periodontal ligament at the furcation lesion of the lower-left-first molar and its surrounding periodontal tissues were observed under a light microscope.

This study was approved by the Matsumoto Dental University Ethics Committee for Animal Experiments (Number #233-13).

Results

Experimental procedure

R_mCT images of the experimental site indicated the occlusal contact position between upper-left-first molar and lower-left-first molar during the experimental period (Fig. 1b). The micro-plus-screwpin did not slip out of the tooth crown, and a decrease of weight was not observed during the experimental term.

Histopathological observations

Throughout the experimental period, no inflammatory reaction was observed at the epithelium around the respective teeth. The down-migration of junctional epithelium was not observed at the cement-enamel junction in any specimens.

In the control, the periodontal ligament maintained a constant width, and main fibers ran across the cementum and the alveolar bone in an orderly manner. The fibroblasts in the periodontal ligament appeared spindle shaped among the fiber bundles (Fig. 2a). On day 4, the periodontal ligament was tightly compressed and in a state of capillary congestion. The densities of deeply dyeing cells in hematoxylin staining with a round-shaped nucleus were increased in the periodontal ligament. A few multinucleated giant cells were observed near the surface of the alveolar bone. Compared with specimens from day 4, the numbers of fibroblasts at day 7 had decreased. Multinucleated giant cells had appeared in the central lesion of the periodontal ligament. Cavities of hard tissue resorption were observed on the surface of both the cementum and the alveolar bone. On day 14, the area of resorption on the surface of the cementum and the alveolar bone accompanied by multinucleated giant cells were expanding rapidly (Fig. 2b).

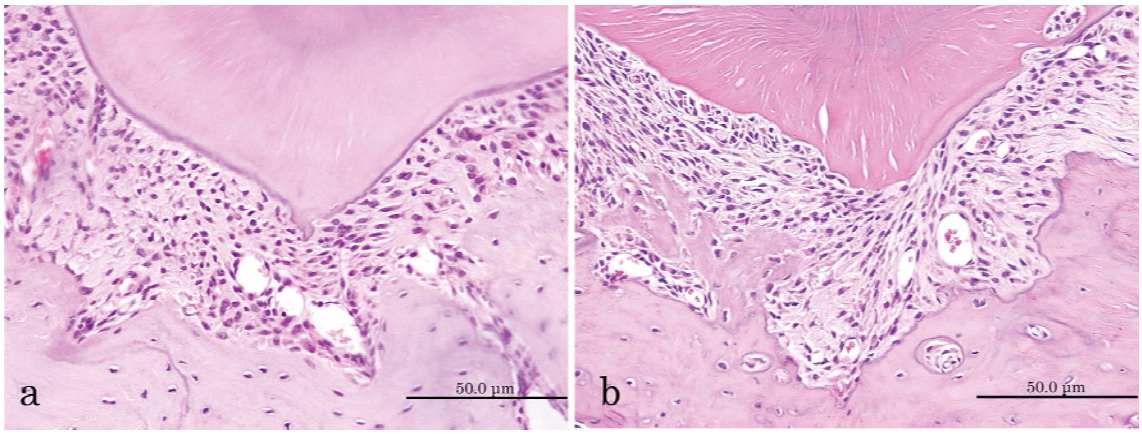

Figure 2. Histopathological photographs of the furcation lesion morphology. a: Control specimen. b: Day-14 specimen. Scale bar = 50.0 μm

Discussion

Experimental models of occlusal trauma

Various experimental designs have been carried out on different animal models with occlusal trauma. Experimental miniature pigs, macaque monkeys, beagle dogs, rabbits and rats have been used

to establish *in vivo* models of occlusal trauma by the way of wrought crowns, casting inlays, or orthodontic square wires attached to the maxillary posterior teeth with methylmethacrylate resin, which lead to the bite raising and occlusal trauma for the opposing mandibular teeth[10,11,13-16]. Several studies have investigated the effect of excessive occlusal load on periodontal tissues. For example, steel wire bonded with methylmethacrylate resin was used to achieve excessive occlusal loading[17]. However, the procedure used in such study was not appropriate in terms of strength and/or period of load, because the bonded wire onto the occlusal surface was able to slip out relatively easily in the humid conditions of the mouth.

We used a standardized micro-plus-screwpin to generate the force-pressure from the excessive occlusal loading in mice. In this study, a micro-plus-screwpin, which placed into the crown cavity, was maintained at a constant position on the occlusal surface throughout the experimental period. Thus, the establishment of a useful experimental model on an extremely small animal, in which the loading is both at a high enough level and is intermittent, is indispensable and allows detailed investigation of periodontal tissues reaction on mechanical loading.

Biological effects on periodontal tissues

It is well known that mechanical loading induces proliferation and differentiation of bone derived cells and extracellular matrix synthesis through various macromolecules[15,18,19]. When a mechanical force is applied to bone, the stress might be detected by certain cells, which then release chemical mediators that induce bone remodeling through autocrine/paracrine mechanisms. These mechano-responsive cells are most likely osteoblasts and osteocytes. Similarly, osteoclasts in the periodontal ligament may play a key role in periodontal tissue breakdown upon excessive occlusal loading.

Previous studies on animal models have described the influence of traumatic occlusion on dental pulp[20,21]. The observations by histological studies showed that the damage to pulp became more serious as time went on, but periodontium appeared to adapt to changes. The results provide experimental evidence for the pathology and pathogeny of pulpitis caused by occlusal trauma. These findings suggest a probable mechanism that could lead to an inflammatory process in the pulp and periapical lesions. From this point of view, we focus our observations on the periodontal ligament of the furcation area of molar teeth, located a short distance from the periapical inflammatory lesion caused by occlusal loading near the apical foramen.

The histological changes of the periodontal ligament at the furcation area of a molar tooth have been observed herein. The present study shows the densities of deeply dyeing cells in hematoxylin staining with a round-shaped nucleus are increased in the periodontal ligament at a peek effect of day 4. Multinucleated giant cells appeared in the central lesion of the periodontal ligament on day 7. The distribution of resorption on the surface of both of the cementum and the alveolar bone accompanied with multinucleated giant cells are expanding rapidly from day 7 to day 14. These findings have showed results approximately similar to those of other studies[10-16].

The present study demonstrates that our *in vivo* model was useful for the detailed investigation of periodontal tissue changes during excessive occlusal loading. Further investigations with occlusal trauma using this experimental system should be considered in the future.

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