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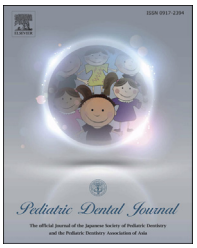

Original Article

Analysis of periapical alveolar bone resorption after the removal of interdental wire ligation

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ABSTRACT

Background/Purpose: Periapical bone resorption is mainly induced by inflammation resulting from an infected root canal. However, chemical and physical stimuli (e.g., traumatic occlusions and excessive orthodontic force) can also cause periapical bone resorption. Here, we report the induction of a periapical bone resorption model without infection in rats.
Methods: Wire ligature was applied between the upper first and second molars for 2 weeks. At 2 weeks and 4 weeks after removal of the wire, the maxillary bones were processed for micro-computed tomography and histological analyses.
Results: The micro-computed tomography analysis showed that periapical bone resorption had occurred and that the bone volume had significantly decreased between Week 2 and Week 4 after wire removal. Histological examination showed a normal apical periodontal ligament. No accumulation of inflammatory cells and no abscess formation were detected in the periapical region. Meanwhile, many tartrate-resistant acid phosphatase-positive osteoclasts were detected in the periapical region of non-wire-ligated rats in which the dental pulp had been exposed; however, active osteoclasts were still located at the alveolar bone in rats even at 4 weeks after wire removal.
Conclusion: These results indicate that induction of periapical bone resorption occurred in the absence of infection and that a bone resorption system different from the infection-associated bone resorption one accounted for the changes.

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1. Introduction

Marginal periodontitis is an infectious disease caused by intraoral periodontal bacteria [1,2]. The disease begins with gingival inflammation and proceeds to gingival recession, the downgrowth of the junctional epithelium toward the root apex, and destruction of the periodontal ligament and alveolar bone. The destruction of the periodontal tissue is aggravated by various factors, including food impaction, occlusal trauma, and various habits [3–5]. In rodents, marginal periodontitis is induced by using a rubber or wire ligature between the first and second molars [6–10]. The recovery of periodontal tissue in rats after removal of the ligature has also been reported [11].

Apical periodontitis is characterized by periapical alveolar bone destruction [12,13] and typically occurs as a deuteropathy of pulpitis and pulp gangrene. Lipopolysaccharides derived from bacteria (e.g., *Streptococcus viridans* and *Streptococcus aureus*) that have invaded the root canal are the primary stimulating factors leading to apical periodontitis [14,15]. However, physical stimulation, such as occlusal trauma and excessive orthodontic force, has also been shown to induce periapical bone resorption [5,16,17].

The pulp exposure method has been widely adopted to analyze apical periodontitis in experimental animal models [16,18]. However, no study has reported a noninfectious system for inducing bone resorption in the area of the root apex. In this study, we report the induction of a noninfectious periapical alveolar bone resorption model in rats. Using this model, we performed a histological and histomorphometric analysis to examine differences in the destruction of periodontal tissue between infectious and noninfectious disease states.

2. Materials and methods

2.1. Animals

Twenty-five 8-week-old conventional female Wistar rats were purchased from Clea Japan (Tokyo, Japan). Because resistance to stress is higher than in males, female rats were used in order to suppress the influence of stress experiments. The experimental protocol used was reviewed and approved by the Animal Care Committee of Showa University, Tokyo, Japan.

2.2. Experimental protocol

After 15 rats were placed under pentobarbital anesthesia, a ligation wire for tooth straightening (0.2-mm diameter; Preformed Ligature Wire; TOMY, Tokyo, Japan) was inserted between the maxillary first and second molar teeth from the buccal to the palatal side and ligated using a needle holder (Fig. 1). The wire was removed after 2 weeks in all rats. Five rats were sacrificed upon removal of the wire (wire-ligation group, $n = 5$). The remaining 10 rats were maintained for 2 weeks (removal group at 2 weeks, $n = 5$) and 4 weeks (removal group at 4 weeks, $n = 5$) prior to sacrifice.

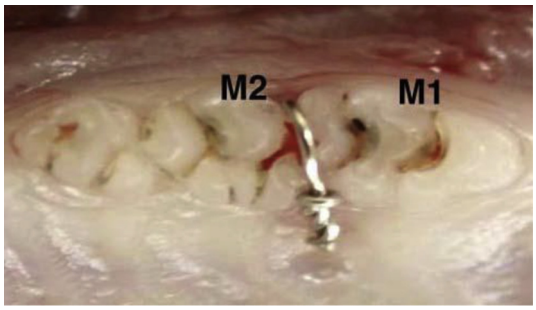

Fig. 1 – Wire ligation between first and the second molars.

To induce infectious periodontitis, the pulps of the right maxillary first molars of five rats were exposed by using a portable, variable-speed electric handpiece (Osada Electric, Tokyo, Japan) and a sterile round burr (exposure group, $n = 5$). The remaining five rats were used as the control group. Two groups were sacrificed at 4 weeks after the treatment.

At the above-indicated periods, the maxillary bones were dissected and fixed with 4% paraformaldehyde in phosphate-buffered saline and processed for the following experiments.

2.3. Micro-computed tomography

Prior to histological preparation, computed tomography (CT) was performed with a micro-CT scanner (inspeXio SMX-90CT microfocus X-ray CT system; Shimadzu, Tokyo, Japan). The maxilla was placed in the sample holder and scanned at the settings of 90 kV and 110 mA, with the voxel size resolution at 0.034 mm. From the obtained datasets, three-dimensional models were built for morphological observation. A line was set between the enamel-cement junction of the first and second molars, and the bone resorption quantity was determined by subtracting the volume of the bone and tooth from the area beneath the line (Fig. 2). Scheffe's test was used for the statistical analysis of the results. A p value <0.05 was considered significant.

After micro-CT analysis, the specimens were decalcified with 10% EDTA, dehydrated with a graded ethanol series, passed through xylene, and embedded in paraffin. Serial sections, 5-μm in thickness, were sectioned and stained with hematoxylin and eosin or Azan solution. Some of the sections were processed for the detection of tartrate-resistant acid phosphatase (TRAP), as described previously [19]. All sections were examined under a light microscope (Axioskop 2 Plus; Carl Zeiss MicroImaging, Göttingen, Germany).

3. Results

3.1. Micro-CT observations

Bone resorption occurred at the buccal alveolar bone border between the first and second molars in the wire-ligation group at 2 weeks, but was not detected in the removal group or the exposure group at 4 weeks (Fig. 2A). In the removal group at 4 weeks, bone resorption was detected around the apical root area and the interalveolar septum between the first and