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Original article

Micro-computed tomography for evaluating alveolar bone resorption induced by hyperocclusion

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ABSTRACT

Purpose: Occlusal trauma, resulting in the destruction of alveolar bone, is a form of periodontal disease caused by excessive mechanical stress (MS) during hyperocclusion. Previously, we showed that C—C chemokine ligand (CCL) 2/CCR2 receptor axis plays a crucial role in MS-dependent osteoclastogenesis. However, in the previous work, we were unable to precisely measure changes in alveolar bone profiles. In the present study, we sought to establish a precise method for evaluating alveolar bone resorption induced by hyperocclusion using micro-computed tomography.
Methods: Under anesthesia, a stainless steel wire was attached to the molars of 5-week-old C57/BL6 wild-type (WT) mice, CCL2$^{-/-}$ mice, and CCR2$^{-/-}$mice to induce occlusal force overload. At days 0 and 7, hard tissue samples were harvested and analyzed by micro-computed tomography.
Results: In the WT mice, bone mineral density of the alveolar bone was significantly decreased at day 7 as compared with day 0, with marked alveolar bone resorption observed. Similarly, significant alveolar bone resorption was observed in the CCL2$^{-/-}$ and CCR2$^{-/-}$ mice at day 7 as compared with day 0.
Conclusions: Micro-computed tomographic images can be used to measure changes in bone mineral density in a mouse model of hyperocclusion. This method may be useful for further investigating bone changes in other periodontal disease research fields.

1. Introduction

Tooth overloading occurs because of premature contact or lateral forces transmitted from partial dentures to abutment teeth, and this excessive mechanical stress (MS) can affect periodontal tissues. Excessive MS and hyperocclusion is referred to as occlusal trauma, a type of periodontal disease. Radiographic findings in patients with occlusal trauma include the loss of the alveolar hard line and enlargement of the periodontal ligament (PDL) space in the early stages, followed by resorption of the alveolar bone and tooth loss at later stages [1,2]. However, normal occlusal forces are necessary, as unloading of teeth can cause extensive alveolar bone remodeling, tooth movement, and even tooth loss [2–4].

We previously developed a hyperocclusal mouse model to study the role of chemokines in osteoclastogenesis in the periodontal ligament. We found that the C—C chemokine ligand (CCL) 2 in PDL tissues played an important role in MS-dependent osteoclastogenesis, acting via the C—C chemokine receptor (CCR) 2 [5]. Intriguingly, we found that, in CCL2$^{-/-}$ and CCR2$^{-/-}$ mice, hyperocclusion causes an increase in the expression of CCL3, a member of another C—C chemokine family known to potentiate osteoclastogenesis. We surmise that CCL3 compensates for the loss of function in the CCL2/CCR2 pathway to retain osteoclastogenesis [6]. However, we used Tartrate-resistant acid phosphatase (TRAP) staining to determine bone changes in these mice, which may not be sufficiently sensitive to measure subtle changes in mineral content in alveolar bone.

Recent research has attempted to analyze alveolar bone changes using micro-computed tomography (micro-CT) [7–9]. Micro-CT has also been used to evaluate orthodontic tooth movement [10,11] and periodontal tissue destruction induced by periodontitis [12,13]. To date, there have been no reports using

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micro-CT to analyze chemokine-mediated, MS-dependent alveolar bone resorption in response to hyperocclusion.

Given that our previous work used a relatively imprecise method to analyze bone mineral changes in our mouse model of hyperocclusion, in the present study, we sought to build on our previous findings and evaluate changes in alveolar bone resorption in our hyperocclusal mouse model using micro-CT analysis.

2. Materials and methods

2.1. Experimental animals

Five-week-old wild-type (WT) C57/BL6 mice were purchased from Kyudo Animals Co., Ltd. (Tosu, Japan). CCL2$^{-/-}$ and CCR2$^{-/-}$ C57/BL6 mice were purchased from Jackson Laboratories (Sacramento, CA, USA). All animals were treated according to ethical regulations for animal experiments defined by the Animal Care Committee of Fukuoka Dental College, Fukuoka, Japan (No. 000233).

2.2. In vivo hyperocclusion model

For each experiment, mice were anesthetized with isoflurane inhalation. To create a hyperocclusion, a stainless-steel wire (diameter, 0.2 mm; length, 3 mm) was adhered to the occlusal surface of the right maxillary molar using methylmethacrylate resin cement (Super-Bond C&B; Sun Medical Co., Ltd., Moriyama, Japan), as previously described [5,6]. To fasten the wire, the enamel was etched with phosphoric acid for 10 s, washed with water, and dried.

2.3. Micro-CT tomography

Mice were sacrificed using an excess of isoflurane anesthesia at 0 and 7 days after surgery (n = 10 in each group on each time point). The mandibular alveolar bone was harvested, and tissues were fixed with 4 % paraformaldehyde for 24 h. Bones were scanned using a Skyscan 1176 micro-CT scanner (Toyo Corporation, Tokyo, Japan) at 9 μm for each slice with a 0.5-mm aluminum filter. The obtained 16-bit TIFF transmission images were converted into 8-bit BMP tomogram images suitable for analysis using the provided software. Photo images were stored directly or after necessary correction using Data Viewer image editing software (Bruker, Kontich, Belgium).

2.4. Image analysis

2.4.1. Bone mineral density (BMD)

Before measurements, images were adjusted so that the first molar was positioned in the vertical plane. Edited images were stored and analyzed using CT-An image analysis software (Bruker). The section with the most complete view of the first molar and alveolar bone was used as the standard section, with 20 additional sections on the buccal and lingual sides used to measure BMD. Regions of alveolar bone surrounding the mesial and distal roots were analyzed.

2.4.2. Alveolar bone resorption levels

Alveolar bone resorption was estimated by measuring the distance from the cementoenamel junction (CEJ) to the alveolar bone crest of the first molar, defined using the following equation: The distance of CEJ-alveolar bone crest (C) = (the distance between CEJ and tip of tooth root [A]) – (the distance between alveolar bone crest and tip of tooth root [B]).

Measurements were performed on each tooth root on the buccal and lingual sides in the coronal plane. A standard section that showed the largest, clear surface for each tooth root was first selected. Then, two sections toward the mesial and distal ends were used for analysis, with a total of five sections analyzed. The mean values from these five sections were used to define alveolar bone resorption.

2.5. Statistical analysis

Data are expressed as the mean and standard error (± SE). Differences in bone mineral density between day 0 and day 7 in WT mice were analyzed with a t-test. Differences in alveolar bone resorption among WT, CCL2$^{-/-}$, and CCR2$^{-/-}$ mice were also analyzed with a t-test. P values less than 0.05 were considered significant.

3. Results

3.1. Evaluation of alveolar bone BMD in WT mice after hyperocclusion

Tomographic images of alveolar bone at day 0 and day 7 after hyperocclusion were obtained using micro-CT. A three-dimensional alveolar bone model was reconstructed from the images using Data Viewer image editing software (Bruker), with the models viewable from multiple directions. Images were corrected and reconstructed so that the axis of the first molar was vertical for analysis (see Section 2 “Methods”). The view from the upper lingual aspect shows that the typical symptoms of alveolar resorption were induced by our hyperocclusion model at day 0 and day 7 (Fig. 1A). The region marked by a dotted line is the area of analysis (Fig. 1B). We found that BMD of the alveolar bone surrounding the first molar was significantly lower at 7 days after hyperocclusion than at day 0 in WT mice (Fig. 1C).

3.2. Evaluation of alveolar bone resorption induced by hyperocclusion in WT, CCL2$^{-/-}$ and CCR2$^{-/-}$ mice

Alveolar bone resorption was estimated by measuring the distance from the CEJ to the alveolar bone crest of the mesial and distal roots of the first molar tooth (Fig. 2A). In WT mice, the distance from the CEJ to the alveolar bone crest was significantly greater at day 7 after hyperocclusion as compared with day 0, indicating that alveolar bone resorption had occurred on the lingual and buccal sides of the mesial root. In contrast, there was no significant difference between day 0 and day 7 in either the buccal or lingual side of the distal root (Fig. 2B). In the CCL2$^{-/-}$ mice and CCR2$^{-/-}$ mice, as in the WT mice, the distance from the CEJ to the alveolar bone crest was significantly greater on the lingual and buccal sides of the mesial root after 7 days of hyperocclusion as compared with day 0 (Fig. 3A, B), with no significant differences in the distances from the CEJ to the alveolar bone crest in the distal root (data not shown).

4. Discussion

In our previous study, we reported that CCL2 expressed in the PDL plays a crucial role in MS-dependent osteoclastogenesis [5]. However, in that study, alveolar bone resorption was evaluated using TRAP-positive cell staining. Recent studies have suggested the utility of micro-CT imaging to analyze bone resorption in an animal periodontitis model to evaluate the degree of alveolar bone resorption [13,14] and in orthodontic tooth movement [10,11]. Micro-CT has also been used to analyze bone profiles in segmental osteotomy studies [15]. We thus hypothesized that micro-CT imaging could provide a more detailed analysis of the alveolar bone loss induced by excessive MS in hyperocclusal mice.

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