To clarify the changes of the mandible and alveolar bone in postmenopausal osteoporosis, we examined the changes of structure in mandible and alveolar bone of ovariectomized (OVX) rats. Twelve female Wistar rats (25-week old) were divided into two groups equally. Nine weeks after ovariectomy, we examined the mandibles of OVX and sham control (Sham) rats by micro-focus computed tomography ($\mu$CT). Bone volume and trabecular thickness in OVX group were significantly less than those in Sham group, while the trabecular separation in OVX group was significantly wider than that in Sham group. Three-dimensional images from the $\mu$CT of the mandible demonstrated a reduced bone volume of the mandibular body in the region of the first molar as well as the inter-radicular septum of the first molar in OVX group. The incisor alveolar bone at the interface between the incisor and the bone marrow in OVX group was significantly thinner than that in Sham group. However, there was no significant difference in the thickness of the medial side of the incisor alveolar bone that did not contain or face the bone marrow. These findings demonstrate that the mandibular bone loss in the OVX rats is predominant adjacent to the bone marrow, suggesting bone loss in postmenopausal osteoporosis occurs mainly at the endosteal surface in mandible. J Jpn Soc Periodontol, 46 : 288–293, 2004.
Key words: alveolar bone, micro-focus computed tomography (μCT), three dimension (3D), osteoporosis, ovariection

Introduction

The alveolar bone connects with the tooth cementum via the periodontal ligament (PDL) to support the tooth, and is subject to continuous remodeling through the influence of mechanical stress produced by mastication. Inappropriate occlusion as well as infection with pathogenic bacteria and subsequent reaction of the host defense system cause destruction of the alveolar bone and other tooth-supporting tissue. This is the essential etiology of periodontal disease. In serious cases, the loss of alveolar bone exceeds the necessary amount for tooth support and results in tooth loss. In this context, maintaining the quantity of the alveolar bone is essential for preservation of tooth function.

In recent years, a number of studies have suggested a positive relationship between osteoporosis and oral bone and/or tooth loss[1-6]. Osteoporosis is a condition characterized by low bone mass and microarchitectural deterioration of systemic bone associated with pain, deformity and fractures in elderly people. Postmenopausal women, as a consequence of ovarian atrophy and the resulting decrease in circulating estrogens, are particularly prone to osteoporosis. Indeed, the fact that postmenopausal women using estrogen retain more of their teeth than those who do not supports the positive association between estrogen deficiency and alveolar bone loss[7-14]. Because the bone loss seen in ovariectionized (OVX) rats shares similarities with that of postmenopausal women, OVX rats have been used as an experimental model of human postmenopausal osteoporosis[12,13]. Therefore, the mandibular changes in OVX rats could be extrapolated to those in humans to some extent. Decreased alveolar bone mass has been reported in OVX rats[14,15]. However, there is some controversy about the behavior of the mandibular bone after ovariection. Some investigators have reported that ovariection does not affect the bone mineral density (BMD) of the mandible[16,17]. Moreover, there have been few reports concerning three-dimensional (3D) changes in the mandible and alveolar bone architecture in OVX rats. Therefore, using micro-focus computed tomography (μCT), we examined the 3D changes in the mandible and alveolar bone of OVX rats.

Materials and methods

Animals and ovariection

Twelve female Wistar rats (25-week old) were used in this study. The rats received food containing 1.17% calcium, 0.91% phosphorus and 80 IU vitamin D₃/100 g (MF: Oriental Yeast, Tokyo, Japan) and water ad libitum. They were divided into two groups equally. Bilateral ovariection was performed under the anesthesia with an intraperitoneal injection of pentobarbital, while control rats underwent a sham operation in which the ovaries were simply touched with forceps via a
dorsal approach incision (Sham). At 9 weeks after the operation, the animals were sacrificed and their mandibles were removed. Principles of laboratory animal care were followed and animal experimentation was in compliance with the “Guidelines for the Care and Use of Laboratory Animals in the Health Sciences University of Hokkaido”.

The mandibles were carefully dissected free of soft tissues and rinsed in distilled water. Following dehydration in ethanol, the mandibles were air-dried at 30°C for 10 days.

**μCT analysis**

For the acquisition of μCT images, a MCT-12505 MF (H) instrument (Hitachi Medical Corporation, Tokyo, Japan) was used. The mandible was mounted on a turntable so that the root canal of the mesial root of the first molar was parallel to the horizontal plane. A total of 80 μCT images were acquired from the mesial end of the first molar in slice increments of 50 μm. All images were transferred to Micro CT Pro software (KGT, Tokyo, Japan), and the area of mineralized tissue was extracted using a global thresholding method.

For quantitative 3D evaluation of the mandibular body and alveolar bone, an image that included as much of the mesio-lingual and buccal root as possible was selected as the standard slice (Fig. 1a). Then the lines A, B, and C on the standard slice were defined as follows: line A passed over the apical ends of mesio-buccal and lingual roots; line B passed over the bony ridge at the end of the masseter muscle insertion on the lateral surface of the mandible and the most lingual end of the incisor; line C runs over the PDL around the inter-radicular alveolar bone and ends on line A. The area between lines A and B is the ROI for the mandibular body, and the area enclosed by lines A and C is that for the inter-radicular septum.

b: The areas by which the thickness of the incisor alveolar bone was examined. The medial alveolar bone was defined as the alveolar bone on the medial side of the incisor and corresponding area to the most lingual end to the most labial end of the incisor pulp (colored yellow). The alveolar bone of the incisor-bone marrow interface is defined as the alveolar bone facing the bone marrow at the lingual side of the incisor (colored red).
Table 1 Effect of ovariectomy on three-dimensional (3D) parameters of the mandible and inter-radicular septum of the first molar in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Mandibular body</th>
<th>Inter-radicular septum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sham</td>
<td>O VX</td>
</tr>
<tr>
<td>Bone volume</td>
<td>%</td>
<td>51.4 ± 4.5</td>
<td>37.3 ± 4.4*</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>mm</td>
<td>0.18 ± 0.02</td>
<td>0.15 ± 0.02*</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>/mm</td>
<td>2.59 ± 0.13</td>
<td>2.28 ± 0.31</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>mm</td>
<td>0.20 ± 0.02</td>
<td>0.29 ± 0.06*</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation (SD) of each group (n=6).
* p<0.01 compared with Sham group.
* p<0.05 compared with Sham group.

Table 2 Effect of ovariectomy on thickness of the incisor alveolar bone at the incisor/bone marrow interface and medial alveolar bone.

<table>
<thead>
<tr>
<th></th>
<th>Incisor/Bone marrow interface</th>
<th>Medial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>O VX</td>
</tr>
<tr>
<td>Thickness</td>
<td>0.19 ± 0.04</td>
<td>0.15 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>O VX</td>
</tr>
<tr>
<td>Thickness</td>
<td>0.24 ± 0.03</td>
<td>0.23 ± 0.02</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation (SD) of each group (n=6).
* p<0.05 compared with Sham group.

Labial end of the incisor pulp (colored yellow in Fig 1b). The alveolar bone of the incisor–bone marrow interface was defined as the alveolar bone facing the bone marrow on the lingual side of the incisor (colored red in Fig 1b). The thickness of the incisor alveolar bone was obtained manually in Micro CT Pro software and calculated in 17 slices including the standard slice; 8 slices mesial and 8 slices distal to the standard slice.

Statistical analysis
Data are presented as the mean and standard deviation (SD) of each group. Comparison between groups was done using unpaired t test.

Results
Quantitative 3D evaluation of the mandible showed that bone volume and trabecular thickness in O VX group were significantly less than those in Sham group. Conversely, trabecular separation in O VX group was significantly wider than that in Sham group (Table 1). In the inter–radicular septum of the first molar, as well as in the mandibular body, bone volume and trabecular thickness were significantly lower in O VX group than in Sham group. The trabecular separation in O VX group was significantly wider than that in Sham group. 3D images of the mandible at the first molar in O VX group demonstrated reduced bone volume in the mandibular body as well as in the inter-
radicular septum (Fig. 2).

The incisor alveolar bone at the incisor–bone marrow interface was significantly thinner in the OVX group than in the Sham group (Table 2). However, there was no significant difference in the thickness of the medial incisor alveolar bone.

Discussion

There have been conflicting opinions about the changes of the rat mandible after ovariectomy\textsuperscript{14–17}. Some investigators have reported that total mandibular BMD does not differ between OVX and control rats\textsuperscript{16,17}. In this study, we demonstrated a decrease of bone volume in the mandibular body in the region of the first molar in OVX rats. Together with the recent report by Kuroda et al.\textsuperscript{18}, who demonstrated the regional differences in the mandibular bone decrease in OVX rats, we assume that the reduction of the trabecular bone occurs in the rat mandible after ovariectomy. The relatively small trabecular region in the mandible and the presence of a large incisor may mask the effect of ovariectomy when total mandibular BMD is being examined in previous studies. Moreover, mechanical stress derived from functional occlusion, preventing bone loss may also make it difficult to detect the changes.

The regional analysis by \(\mu\)CT in this study confirmed the decrease of bone volume in the mandible after ovariectomy.

The present study demonstrated a significant decrease of bone mass in the alveolar inter-radicular septum of the first molar in OVX rats. This finding is consistent with the changes in the inter–radicular septum of OVX rats reported by Tanaka et al.\textsuperscript{14,15}. At the same time, the 3D images we obtained showed a decrease in trabecular bone in the mandibular body as well as the inter-radicular septum of the first molar. These results are equivalent to those of previous studies of the femur after ovariectomy\textsuperscript{19,20} in that trabecular bone is affected more than cortical bone. Miyaura et al.\textsuperscript{21} reported that osteoclastic bone resorption in bone marrow was stimulated by interleukin (IL) \(\text{o}-6\) and IL-\(\text{I}\) when estrogen was deficient. A similar mechanism may have been involved in the trabecular bone loss in the mandibular body and inter–radicular septum observed in this study.

Liu and Baylink\textsuperscript{22} demonstrated differences in the response of osteoclasts located in the endostem and bony socket facing the incisor when the systemic mineral content was changed, suggesting a divergent mechanism of bone metabolism in bone enclosing bone marrow and alveolar bone. Indeed, in our present analysis of the thickness of the incisor alveolar bone, the alveolar bone between the incisor and bone marrow in OVX rats was significantly thinner than that in Sham rats. On the other hand, the thickness of the medial incisor alveolar bone did not differ significantly between the two groups. The fact that the medial incisor alveolar bone did not contain or face the bone marrow and resembled the cortical bone of long bones suggests that the endosteal surface is the predominant area of the bone that responds to the signal produced by systemic factors such as changes in mineral content and hormones.

(The 108th Annual Meeting of the Japanese Association of Anatomists)

References

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