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Mineralized bone loss in partially edentulous trabeculae of ovariectomized rabbit mandibles

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Objective: The purpose of this study was to evaluate trabeculae changes in partially edentulous bone in ovariectomized rabbits.

Background: Numerous clinical studies have suggested that the greater risk for oral bone loss in females may be correlated with osteoporosis after menopause. Knowledge of trabecular changes in partially edentulous bone in animals with loss of ovarian function may be beneficial in the diagnosis and treatment of partially edentulous patients of postmenopausal women.

Methods: Twelve adult female Japanese white rabbits were examined. The mandibular incisors were initially extracted to simulate the partially edentulous bone. Six animals were bilaterally ovariectomized and the other six sham-ovariectomized 12 weeks after tooth extraction. The partially edentulous parts of distal mandibular bodies were processed undecalcified 12 weeks after ovariectomized or sham-ovariectomized surgeries and examined by quantitative trabecular bone histomorphometry.

Results: In ovariectomized rabbits, there were significant increases in trabecular separation, osteoid volume, osteoid thickness, osteoid width, eroded surface, and mineral apposition rate, and a significant decrease in trabecular number.

Conclusion: The results of sparser trabecular structure, more trabecular osteoid, and increased trabecular bone turnover demonstrate mineralized bone loss in partially edentulous trabeculae of ovariectomized rabbit mandibles and suggest that the same loss may occur in postmenopausal women.

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Osteoporosis is a morbid clinical condition of ill-defined pathogenesis in which there is progressive and inexorable loss of skeletal mass, rendering bone increasingly vulnerable to fracture (1). In addition to osteoporosis, loss of bone from the oral skeleton or the maxillofacial complex also significantly impacts the health and quality of life of patients, especially with advancing age, affecting up to 94% of elderly individuals (2). There is now significant interest in the interrelationship of systemic osteoporosis to oral bone loss (3–9). A recent study in the dentulous mandibles of ovariectomized rats indicated that estrogen deficiency caused osteoporotic changes and thin alveolar proper in the interradicular septum of first molar (10). Our previous study with peripheral computed tomography suggested mineralized bone loss in the edentulous part of mandibles of ovariectomized rabbits, especially in the trabecular bone region (11). Detailed knowledge of trabecular changes in the partially edentulous bone with loss of ovarian function may be beneficial in the diagnosis and treatment

of partially edentulous patients of postmenopausal women. The purpose of this study was to evaluate the trabeculae histomorphometric changes in partially edentulous bone of ovariectomized rabbit mandibles.

Materials and methods

Animals

Adult female Japanese white rabbits ($n = 12$, average weight 3.5 kg; Japan Clea Co. Ltd, Tokyo, Japan) were obtained at 12 weeks of age and allowed to acclimate for 1 week. The rabbits were housed in individual cages and maintained in a controlled environment at 24 ± 1°C, humidity 55 ± 5%, with a circadian light rhythm of 12 h. Standard laboratory chow (RC4, Oriental Yeast Co., Chiba, Japan) and water were available ad libitum to the rabbits. Animal experiments were approved by the Laboratory Animal Committee, Showa University.

Experimental design

The bilateral mandibular incisors of each animal were initially extracted and the maxillary incisors were cut at the gingival edge under anesthesia [pentobarbital sodium (Abbott Laboratories, North Chicago, IL, USA), 25 mg/kg body weight, intraperitoneal injection]. Twelve weeks after tooth extraction, bilateral ovariectomized surgeries were performed on six of the rabbits using the same anesthesia conditions. sham-ovariectomized surgeries were performed on the other rabbits, as a control. For the sham-ovariectomized operation, the ovaries were held up and then returned to their original position. All animals were given intraperitoneal injections of calcein (4 mg/kg body weight; Wako Pure Chemical Industries, Ltd, Osaka, Japan) and intramusclar injections of tetracycline hydrochloride (20 mg/kg; Wako Pure Chemical Industries, Ltd) on days 7 and 2 before necropsy; both are used as fluorochrome bone markers for histomorphometry. All rabbits were killed at 12 weeks after ovariectomized or sham-ovariectomized operation.

Histologic preparation

The right mandibles were collected, then fixed and stained for 15 days in Villanueva's bone stain solution (Polysciences, Inc., Warrington, PA, USA). They were then dehydrated in ethanol and embedded undecalcified in polyester resin (Nisshin EM Co., Ltd, Tokyo, Japan). Horizontal sections from the middle level of incisor-extracted mandibular parts were cut with a low-speed bone saw (Maruto Co., Ltd, Tokyo, Japan). Sections were mounted on plastic slides, cut and ground to approximately 15 μm in thickness with an Exakt cutting-grinding system (Exakt, Hamburg, Germany), then polished and covered with micro cover glasses.

Quantitative trabecular bone histomorphometry

The partially edentulous trabecular region of the section was measured using a MGA-4300 digitizing Image Analyzing System (CSS Co., Ltd, Nagano, Japan), interfaced with a light and fluorescence microscope (Eclipse E600; Nikon, Tokyo, Japan). The region located within one cortical width from the endocortical surface was excluded from the measurements (Fig. 1). For general bone structure, the parameters of trabecular bone volume (BV/TV, %: bone volume/total volume), trabecular thickness (Tb.Th, μm: average trabecular thickness), trabecular number (Tb.N, mm^{-1}: trabecular numbers per square millimeter) and trabecular separation (Tb.Sp, μm: average distance between trabeculae) were measured and calculated. For osteoid structure, relative osteoid volume (OV/BV, %: osteoid volume/bone volume), absolute osteoid volume (OV/TV, %: osteoid volume/total volume), osteoid surface (OS/BS, %: osteoid surface/bone surface), osteoid thickness (O.Th, μm: average osteoid thickness), and mean osteoid width (OV/OS, μm: osteoid volume/osteoid surface) were measured and calculated. For bone resorption, eroded surface (ES/BS, %: eroded surface/bone surface) was measured; resorption lacunar surface of the trabeculae was identified as eroded surface. For bone formation, the following parameters were measured and calculated: single-labeled surface (sLS/BS, %: single-labeled surface/bone surface), double-labeled surface (dLS/BS, %: double-labeled surface/bone surface), mineralizing surface (MS/BS, %: mineralizing surface/bone surface), mineral apposition rate (MAR, μm/day), and bone formation rate [BFR/BS, $\mu m^3/(\mu m^2$ day): bone formation rate/bone surface].

Statistical analysis

Results were expressed as the mean ± standard error of the mean. The indices of histomorphometry were compared by two-tailed *t*-test analysis of variance between the sham-ovariectomized and ovariectomized groups. *p*-values less than 0.05 were considered significant.

Results

All rabbits remained healthy throughout the experimental periods. The success of the ovariectomization was confirmed at necropsy by

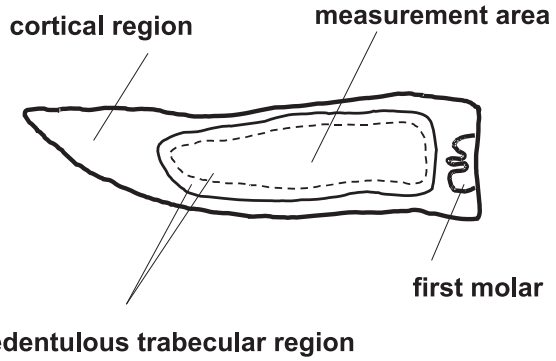

Fig. 1. Measurement area in middle horizontal section from incisor-extracted part of ovariectomized rabbit mandible.

marked atrophy of the uterine horns and no histological evidence of ovarian tissue at the surgical site. Substantial atrophy of the uterus horns confirmed the reduction of serum estrogen levels in the ovariectomized animals (12).

General trabecular structure

Histological sections showed a sparser edentulous trabecular structure in the mandibles of ovariectomized animals, compared with that of sham-ovariectomized animals (Fig. 2). The histomorphometric indices of general trabecular structure are presented in Table 1. In trabecular bone volume and trabecular thickness indices, there was no significant difference between the ovariectomized and sham-ovariectomized animals. In ovariectomized animals, there was a significant decrease in trabecular number and a significant increase in trabecular separation.

Trabecular osteoid structure

The histomorphometric indices of osteoid structure are presented in Table 2. There were significant increases in relative osteoid volume, absolute osteoid volume, osteoid thickness, and mean osteoid width in ovariectomized animals. There was no significant increase in osteoid surface in ovariectomized animals.

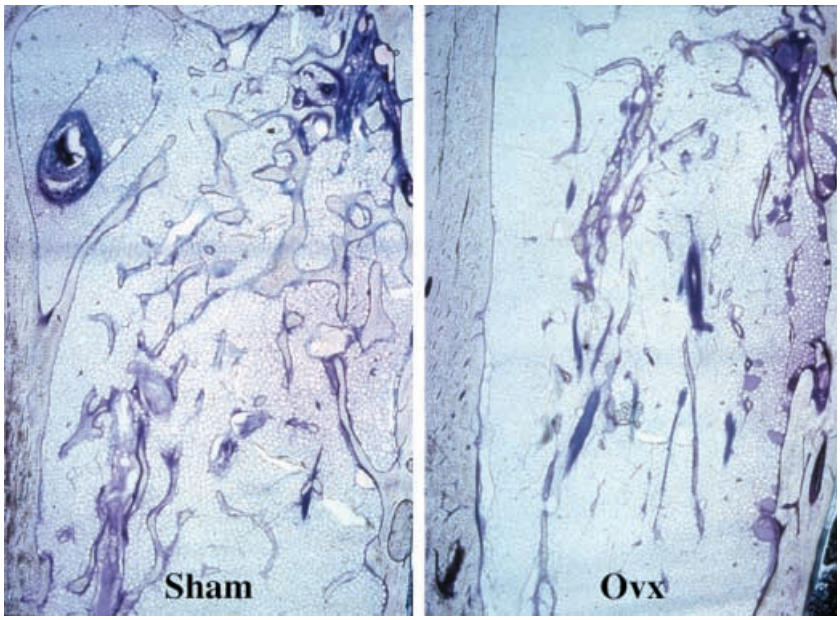

Fig. 2. Trabecular structure of partially edentulous bone of ovariectomized (Ovx) and sham-ovariectomized (Sham) rabbits mandibles (Villanueva's bone stain, original magnification ×10).

Table 1. General trabecular structure of partially edentulous bone of sham-ovariectomized (Sham) and ovariectomized (Ovx) rabbit mandibles

	Sham	Ovx
Trabecular bone volume (BV/TV, % ± SE)	9.687 ± 0.775	6.761 ± 1.285
Trabecular thickness (Tb.Th, μm ± SE)	83.864 ± 7.029	76.057 ± 11.976
Trabecular number (Tb.N, mm^{-1} ± SE)	1.157 ± 0.030	0.868 ± 0.079[b]
Trabecular separation (Tb.Sp, μm ± SE)	782.378 ± 21.766	1145.136 ± 115.947[a]

BV/TV: bone volume/total volume; Tb.Th: average trabecular thickness; Tb.N: trabecular numbers per square millimeter; Tb.Sp: average distance between trabeculae.
[a]Significantly different from Sham group, $p < 0.05$.
[b]Significantly different from Sham group, $p < 0.01$.

Trabecular bone resorption

The histomorphometric indices of bone resorption are presented in Table 3. There was a significant increase in eroded surface in ovariectomized animals.

Trabecular bone formation

The histomorphometric indices of trabecular bone formation are presented in Table 4. In single-labeled surface, double-labeled surface, mineralizing surface indices, and bone formation rate, there was no significant difference between the ovariectomized and sham-ovariectomized animals. In ovariectomized animals, there was a significant increase in the mineral apposition rate.

Discussion

Our previous study with peripheral computed tomography suggested significant decrease in bone mineral density of trabecular area in partially edentulous mandibles of ovariectomized rabbits, whereas no such decrease in cortical area of the same mandibles (11). We applied histomorphometry in the current study to investigate the detailed changes in trabecular area in partially edentulous mandibles of ovariectomized rabbits. The results of this study demonstrate significant structural changes in trabecular number and separation of partially edentulous bone of ovariectomized rabbit mandibles. A decrease in trabecular number and an increase in trabecular separation resulted in a sparser edentulous trabecular structure in mandibles following ovariectomy (Fig. 2). Although the decreases in trabecular bone volume and trabecular thickness were not significant, marked increases in relative osteoid volume, absolute osteoid volume, osteoid thickness, and mean osteoid width revealed a substantial increase in osteoid and a relative decrease in mineralized bone in the partially edentulous trabeculae following ovariectomy. The substantial increases in trabecular osteoid indices also demonstrate a marked increase in

Table 2. Trabecular osteoid structure of partially edentulous bone of sham-ovariectomized (Sham) and ovariectomized (Ovx) rabbit mandibles

	Sham	Ovx
Relative osteoid volume (OV/BV, % ± SE)	0.220 ± 0.079	1.328 ± 0.407[a]
Absolute osteoid volume (OV/TV, % ± SE)	0.020 ± 0.007	0.069 ± 0.013[b]
Osteoid surface (OS/BS, % ± SE)	5.573 ± 1.912	10.376 ± 2.252
Osteoid thickness (O.Th, μm ± SE)	1.280 ± 0.142	4.142 ± 0.472[d]
Mean osteoid width (OV/OS, μm ± SE)	1.499 ± 0.062	4.079 ± 0.603[c]

OV/BV: osteoid volume/bone volume; OV/TV: osteoid volume/total volume; OS/BS: osteoid surface/bone surface; O.Th: average osteoid thickness; OV/OS: osteoid volume/osteoid surface.
[a]Significantly different from Sham group, $p < 0.05$.
[b]Significantly different from Sham group, $p < 0.01$.
[c]Significantly different from Sham group, $p < 0.005$.
[d]Significantly different from Sham group, $p < 0.0005$.

Table 3. Trabecular bone resorption in partially edentulous bone of sham-ovariectomized (Sham) and ovariectomized (Ovx) rabbit mandibles

	Sham	Ovx
Eroded surface (ES/BS, % ± SE)	5.865 ± 0.917	15.287 ± 1.499[a]

ES/BS: eroded surface/bone surface.
[a]Significantly different from Sham group, $p < 0.001$.

Table 4. Trabecular bone formation in partially edentulous bone of sham-ovariectomized (Sham) and ovariectomized (Ovx) rabbit mandibles

	Sham	Ovx
Single-labeled surface (sLS/BS, % ± SE)	16.847 ± 6.177	17.885 ± 4.469
Double-labeled surface (dLS/BS, % ± SE)	9.330 ± 3.161	10.612 ± 2.294
Mineralizing surface (MS/BS, % ± SE)	17.754 ± 4.275	19.555 ± 2.879
Mineral apposition rate (MAR, μm/day ± SE)	0.651 ± 0.089	1.061 ± 0.045[a]
Bone formation rate [BFR/BS, $\mu m^3/(\mu m^2$ day) ± SE]	0.039 ± 0.013	0.075 ± 0.011

sLS/BS: single-labeled surface/bone surface; dLS/BS: double-labeled surface/bone surface; MS/BS: mineralizing surface/bone surface; MAR: mineral apposition rate; BFR/BS: bone formation rate/bone surface.
[a]Significantly different from Sham group, $p < 0.05$.

new bone formation in the partially edentulous trabeculae following ovariectomy.

An approximately three-fold increase in the trabecular bone resorption index of the eroded surface was observed in ovariectomized rabbits compared with the sham-ovariectomized rabbits. Meanwhile, a greater trabecular bone formation index of mineral apposition rate was observed in the ovariectomized rabbits, even though the increases in the other trabecular bone formation indices were not significant. This increase in the mineral apposition rate is indicative of an increase in osteoblastic activity at the cellular level (13). The simultaneously increased resorption of existing trabecular bone and increased formation of new trabecular bone were manifest as increased bone turnover, resulting in new trabecular bone that was more osteoid but less mineralized, at least initially, than the older trabecular bone that was resorbed (14). This increased bone turnover in sparser trabeculae with more osteoid (increase in relative osteoid volume, absolute osteoid volume, osteoid thickness and mean osteoid width) indicates a net loss of mineralized bone in partially edentulous trabeculae following ovariectomy in rabbits. It is identical with that of recent studies on the mandibular condyles and dentulous alveoli of ovariectomized rats (9, 10) and confirms our previous study on mandibles of ovariectomized rabbits (11). The results of this study suggest that mineralized bone loss may occur in the partially edentulous trabeculae of postmenopausal patients and careful examination and suitable treatment are needed for these patients.

Many researchers have focused on ovariectomized animal models for studying the clinical outcome of postmenopausal bone loss (15–20). Rats have been the most widely used animal species (21). Rabbits achieve skeletal maturity shortly after reaching complete sexual development, unlike other mammals such as the rat, mouse, and guinea pig (22). Other cited advantages of rabbits include a shorter developmental period and faster bone turnover than bigger mammals, such as primates (22). The availability, ease and adaptability to experimental manipulation of the rabbit also make this animal a potentially appropriate animal model for studies of osteoporosis and oral bone loss. This study also suggests that an ovariectomized rabbit with the mandibular incisors extracted is suitable for the study of partially edentulous trabecular bone with loss of ovarian function.

In summary, this study documents the general trabecular structure, osteoid structure, trabecular resorption, and trabecular formation changes in partially edentulous bone of ovariectomized rabbit mandibles. The results of sparser trabecular structure, more trabecular osteoid, and increased trabecular bone turnover demonstrate mineralized bone loss in the partially edentulous trabeculae of ovariectomized rabbit mandibles and suggest the same loss may occur in postmenopausal women.

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