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Original

Prevention of trabecular bone loss in the mandible of ovariectomized rats

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Abstract: The effect of therapeutic agents on trabecular bone loss in the mandible was investigated in ovariectomized rats. Eighty-seven Wistar SPF female rats were ovariectomized (OVX) or given a sham operation (Sham), and maintained on a diet containing 0.1% calcium. Four weeks later, groups of OVX rats were treated with estriol (E3), calcitonin (CT), etidronate, or 2-carboxyethylgermanium sesquioxide (Ge-132). The Basal group was maintained on a diet containing 1.0% calcium, and the OVX and sham groups on a diet containing 0.1% calcium. The trabecular bone mineral density (BMD) and trabecular bone mineral content (BMC) in 11 mandibular slices from 0.5 mm at the mesial margin of the first molar to 0.5 mm at the distal margin of the third molar, were measured using peripheral Quantitative Computed Tomography (pQCT). The BMD in the OVX group was lower than that in the Sham group, and decreased BMC was observed only in the molar region. BMD and BMC were increased in the etidronate-treated group, but only BMC was increased in the CT group. E3 treatment increased BMD and BMC; significant increases were also observed beneath the molar. Ge-132 treatment increased both BMD and BMC, especially the latter. (J. Oral Sci. 46, 75-85, 2004)

Key words: mandible; trabecular BMD and BMC; ovariectomized rats; therapeutics; pQCT.

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Introduction

A large cohort of postmenopausal women who show systemic bone loss and osteoporosis also carry a high risk of tooth and oral bone loss (1-3). Such tooth loss, serious alveolar bone resorption, and periodontal disease may be associated with loss of mandibular bone mineral content. It is well known that post-menopausal estrogen deficiency causes substantial bone loss in long bones and vertebrae, although bone loss in the mandible is not well defined. Unlike the case of long bones, the mandible is a kind of membranous bone with the exception of its condyle. The content (volume) of trabecular bone in the mandible is considerably lower than that of cortical bone. This suggests that precise quantitative determination of trabecular bone loss and damage in the mandible due to systemic osteoporosis would be difficult.

The function of the mandible is to transfer occlusal force to bone through the teeth. Because the trabecular bone within the compact mandible is surrounded by the tooth socket, it supports the teeth and plays an important role in the transfer and distribution of stress. Furthermore, it also possesses an important function in bone grafting and implantation to maintain local bone mass in the mandible. Thus, it is necessary to clarify the changes occurring in trabecular bone in the mandible during the postmenopausal period.

There have been a few reports on the osteoporotic effect of estrogen deficiency on the mandible. These studies have suggested that loss of mandibular bone mineral content (BMC) in postmenopausal women is generally correlated with lumbar vertebral BMC (4) and that mandibular bone mass is strongly correlated with systemic bone mass (5). Daniell (6) has reported that the probability

of edentia is approximately three times higher in women with severe osteoporosis than in normal women, and that the major cause of tooth loss is alveolar bone resorption, due to systemic osteoporosis (7). Klemetti et al. (8) have demonstrated that systemic osteoporosis results in a substantial decrease of cortical BMD in the mandible of postmenopausal women, but does not result in decreased trabecular BMD. In contrast, it has been shown that loss of mandibular BMD in postmenopausal women is not correlated with BMD in the lumbar vertebrae and femur (9) and that systemic osteoporosis does not affect the mandible when sufficient occlusal force is present (10). These discrepant findings suggest that the effect of systemic osteoporosis on mandibular bone loss has not been well defined, because of the difficulties inherent in studying postmenopausal bone loss in the mandible and a number of confounding variables.

On the other hand, it has been reported that the osteoporosis due to estrogen deficiency can be prevented by treatment with drugs. Wang et al., (11) demonstrated that treatment with parathyroid hormone following ovariectomy (OVX) significantly increased both trabecular and cortical BMD and BMC as well as substantially increasing the bone mass of the L3 vertebral bone in female rats. Calcitonin (CT) is widely used in the treatment of osteoporosis because of its inhibitory effect on bone resorption. Shen et al. (12) showed that CT treatment depressed bone turnover and provided complete protection against moderate cancellous osteopenia in the femoral neck of OVX rats. Mochizuki and Inoue (13) have reported that CT had an inhibitory action on bone resorption as well as being effective for maintaining and stimulating bone formation in vivo in rats with experimental osteoporosis induced by OVX and a low-calcium diet. A recent study (14) in OVX rats showed that CT provided an important alternative therapy for postmenopausal osteoporosis. Estriol (E3) is one of the three active estrogens found in the body. The major application of E3 therapy is hormone replacement to delay bone loss after the menopause. In particular, E3 seems to be applicable to aged women because it has a much weaker stimulating effect on the breast and uterine lining than estradiol (E2) and estrone (E1). For example, E2 is 1000 times more stimulatory to breast tissue than E3. An early study (15) in OVX rats showed that E3 significantly improved BMD of the femur and tibia by inhibiting bone resorption, accompanied by a less stimulatory effect on the breast and uterine lining.

Etidronate is a potent anticalcifying agent used for the treatment of bone resorption, hypercalcemia and osteoporosis. The bone mass-preserving action of etidronate has been well documented in OVX rats (16). Recently, studies (17,18) in OVX rats have shown that etidronate treatment not only maintained BMD and trabecular bone in the femur and proximal tibia, but also maintained their structure and mechanical properties. The beneficial effect of etidronate in the prevention of bone loss was confirmed in postmenopausal women (19). Cyclical administration of this agent has also been shown to preserve the trabecular structure of human bones without negatively affecting bone mineralization (20). In addition, the therapeutic efficacy of this agent for treating various kinds of osteopenia, including postmenopausal, senile, and corticosteroid-induced osteoporoses, is of current interest. In contrast, reports on the effect of 2-carboxyethyl-germanium sesquioxide (Ge-132) treatment on osteoporosis in OVX rats have been relatively few. Orimo et al. (21) demonstrated that Ge-132 enhanced osteoblast activity. Naitou (22) reported that the bone resorptive activity of newborn mouse calvaria was significantly inhibited by administration of Ge-132. Our previous studies (23,24) have also demonstrated that Ge-132 had a beneficial effect on femoral bone mass in OVX rats.

Our recent study (25) of OVX rats fed a low-calcium diet showed that bone loss occurred in the mandible accompanied by systemic osteoporosis. However, the effects of the above agents on mandibular bone loss in OVX rats remain uncertain. Therefore, there has been a need to carry out further studies of changes in the trabecular bone of the mandible and to clarify the effects of treatment on these changes in an estrogen deficiency-induced systemic osteoporosis model. In the present study, changes in the mandible in OVX rats with osteoporosis and the therapeutic effect of various agents were examined. Trabecular BMD and BMC were measured in 11 mandibular slices, from 0.5 mm at the mesial margin of the first molar to 0.5 mm at the distal margin of the third molar, using peripheral quantitative computed tomography (pQCT). The correlation of the data obtained with the measured slice positions was discussed.

Materials and Methods

Animals

Ninety-nine Wistar strain SPF female rats, 11 weeks old with an average body weight of 160 g, were obtained from Japan SLC (Shizuoka, Japan). The rats were maintained on a 1% calcium diet (Oriental Yeast, Tokyo, Japan), and weighed once a week. Throughout the experiment, the rats were kept at a room temperature of 23 ± 1°C and 60 ± 10% humidity under a 12-hour light/dark regime (lights on 08:00-20:00). All animals were maintained and used in accordance with the guide for the Care and Use of Laboratory Animals of Nihon University,

School of Dentistry at Matsudo. At 26 weeks of age, 77 rats were anesthetized using 35 mg/kg pentobarbital i.p., and then subjected to OVX. Twelve rats were sham-ovariectomized (Sham), i.e., the ovaries were exposed but not removed. The OVX and Sham rats were maintained on a 0.1% calcium diet (modified AIN-93G, Oriental Yeast, Tokyo, Japan). The Basal group of twelve rats was further maintained on a 1.0% calcium diet to compare the effect of the low-calcium diet on osteoporosis.

Experimental Protocol

In order to examine therapeutic effects against trabecular bone loss in OVX rats, the rats were divided into the following treatment groups at 4 weeks after OVX (Fig. 1):

(1) OVX with estriol (E3) treatment (OVX + E3): Fifteen OVX rats were given estriol (Teikoku Hormone Mfg., Tokyo, Japan) orally at a dose of 100 μg/kg/day, 5 days a week, for 12 weeks.

(2) OVX with calcitonin (CT) treatment (OVX + CT): Fifteen OVX rats were given calcitonin (Teikoku Hormone Mfg., Tokyo, Japan) subcutaneously at a dose of 8 U/kg/day, 5 days a week, for 12 weeks.

(3) OVX with etidronate treatment (OVX + Etidronate): Fifteen OVX rats were given etidronate (Sumitomo Medicine Manufacture, Tokyo, Japan) subcutaneously at a dose of 5 mg/kg/day, 5 days a week, for 2 weeks.

(4) OVX with poly-trans-(2-carboxyethyl) germaniumsesquioxide (Ge-132) treatment (OVX + Ge-132): Fifteen OVX rats were given Ge-132 (CAS, 2-carboxy-ethylgermanium sesquioxide, Asai Germanium, Tokyo, Japan) orally at a dose of 108 mg/kg/day, 5 days a week, for 12 weeks.

(5) OVX control (OVX): Fifteen OVX rats served as the controls.

At 42 weeks of age, the rats were sacrificed and the mandible was extracted. Soft tissue surrounding the bone was removed and the mandible was immersed in 70% ethanol.

Analysis of Mandibular Bone

The trabecular BMD and BMC of the mandible were measured using a pQCT system (XCT Research SA, Stratec, Medizintechnik, GmbH) with software version XCT-540. The mandible was positioned so that the scanning arm moved in a direction perpendicular to the occlusal plane. Each mandible was scanned, using a voxel size of 100 μm and a SV scan speed of 10 mm/s. Eleven slices, 0.75 mm apart, from 0.5 mm at the mesial margin of the first molar to the distal margin of the third molar, were obtained (Fig. 2a). In order to accurately separate the cortical bone from the trabecular bone during pQCT measurement, the corresponding image of the undecalcified mandibular slice was obtained using a soft X-ray fluoroscope (Softex Type SRO-M40) prior to the measurement (Fig. 2b). By using the soft X-ray image, the mandibular region of interest (ROI), which did not contain the molar tooth (crown and root), incisor (root) or cortical bone, was used to analyze the trabecular BMD (Fig. 2c).

pQCT has been used to measure cortical and trabecular BMDs and BMCs for the last decade, focusing particularly on long bones and vertebrae in rats and humans (26,27). In these studies an attenuation threshold and the settlement of the area ratio were used to separate cortical bone from

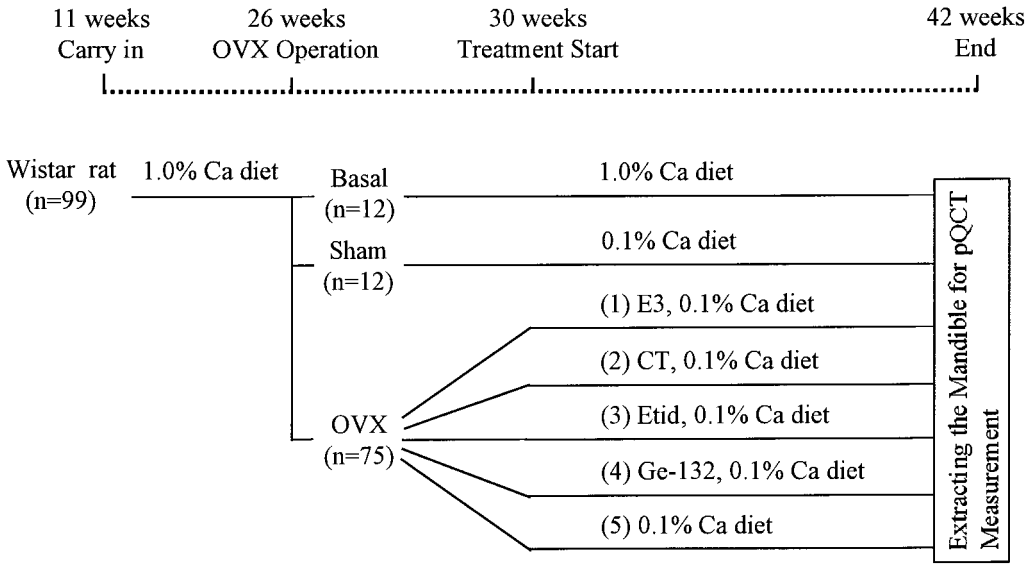

Fig. 1 Protocol for the experiment.

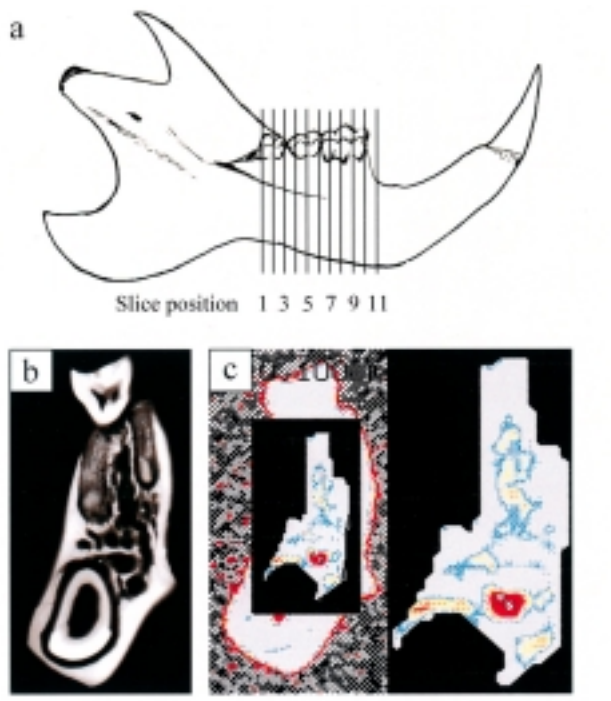

Fig. 2 a: Schema of the mandibular bone with the landmarks used for measurement; b: soft X-ray photograph of a slice underneath the first molar (Sham rat); c: pQCT image of a slice underneath the first molar (Sham rat).

trabecular bone (26,27). However, due to the dense distribution of bone in the mandible compared with long bones, our study (25) suggested that the method might not be applicable to the mandible. This is due to the fact that the BMD of trabecular bone in the mandible is much higher than that of cortical bone, and also the complex geometry of the mandible, resulting in greater measurement error. Therefore, in the present study ROI density analysis was applied to separate cortical from trabecular bone in the rat mandible instead of the conventional attenuation threshold and the settlement of the area ratio.

Table 1 Comparison of average body weight during the experimental period

Groups	OVX Operation (26 weeks)	Start of Treatment (30 weeks)	End of Experiment (42 weeks)
Basal	213.7 ± 12.4	224.8 ± 13.3 $^{\oslash}$	241.5 ± 17.2 *
Sham	203.5 ± 9.3	207.5 ± 9.9 $^{\#\Delta}$	248.1 ± 22.1 $^{\#}$
OVX	203.1 ± 11.4	223.1 ± 11.5 $^{\kappa}$	275.4 ± 19.2 $^{\kappa\Pi}$
Estriol (E3)	206.6 ± 10.1	232.2 ± 19.6 $^{\Theta}$	230.2 ± 17.2*
Calcitonin (CT)	208.6 ± 10.0	237.6 ± 13.1 $^{\Theta}$	273.4 ± 16.4 $^{\kappa\Pi}$
Etidronate	213.2 ± 8.9	242.4 ± 12.5 $^{\Theta\&\Delta}$	298.4 ± 22.8 $^{\Theta\&\Pi}$
Ge-132	205.6 ± 11.0	232.8 ± 22.8 $^{\Theta}$	282.8 ± 28.0 $^{\Theta\Pi}$

Data are presented as the mean ± SD (n = 12-15).
Difference from Sham: $^{\oslash}$ $P < 0.05$; $^{\kappa}$ $P < 0.01$; $^{\Theta}$ $P<0.001$.
Difference from OVX Control: $^{\&}$ $P < 0.05$; $^{\#}$ $P < 0.01$; * $P < 0.001$.
Difference from Basal: $^{\Delta}$ $P < 0.05$; $^{\Pi}$ $P < 0.001$.

Statistical Analysis

Statistical analysis was performed using Statistica Version 5J (Original, Stat Soft, USA). The statistical significance of differences between mean values in the three groups was assessed by analysis of variance (ANOVA), and multiple-comparison tests (MANOVA) were performed using the Newman-Keuls test (Original, Stat Soft, USA). Differences at $P < 0.05$ were considered significant.

Results

Body Weight

The average body weight of each group during the experimental period is shown in Fig. 3 and Table 1. There was no substantial difference in average body weight before OVX (at 26 weeks old). However, the body weight of the OVX group was greater than that of the Sham group, whose body weight in turn was nearly the same as that of the Basal group throughout the experimental period. The body weight of estriol-treated rats decreased sharply, as a result of its side effect. For calcitonin-treated rats, the body weight decreased during the first week of medication and then remained nearly constant; however, it increased significantly beyond 12 weeks after OVX. Etidronate and Ge-132 treatments significantly increased body weight. The maximum increase in body weight was seen in the etidronate-treated rats.

Trabecular BMD

The changes in the trabecular BMD of the mandible in each group are summarized in Fig. 4. The BMD differed considerably among the measured slice positions. Trabecular BMD was lower in the Sham group than in the Basal group; a particularly substantial decrease of trabecular BMD was observed in slices 2, 3, 4 and 7. Furthermore, the trabecular BMD was much lower in the OVX group than in the Sham group in all slices. A greater increase of trabecular BMD was seen in the estriol, calcitonin and Ge-132 groups in many slices compared with the OVX group. The trabecular BMD in the etidronate-treated group was higher than that in the OVX group, and also higher than that in the Sham group in the molar region (slices 4, 7, 10 and 11).

Trabecular BMC

The changes in the trabecular BMC of the mandible in each group are summarized in Fig. 5. The trabecular BMC also varied according to the measured slice position, and decreased monotonously from the first to the third molar. A significantly greater loss of trabecular BMC in the mandible was observed in all slices in the Sham group than in the Basal group. On the other hand, a more significant

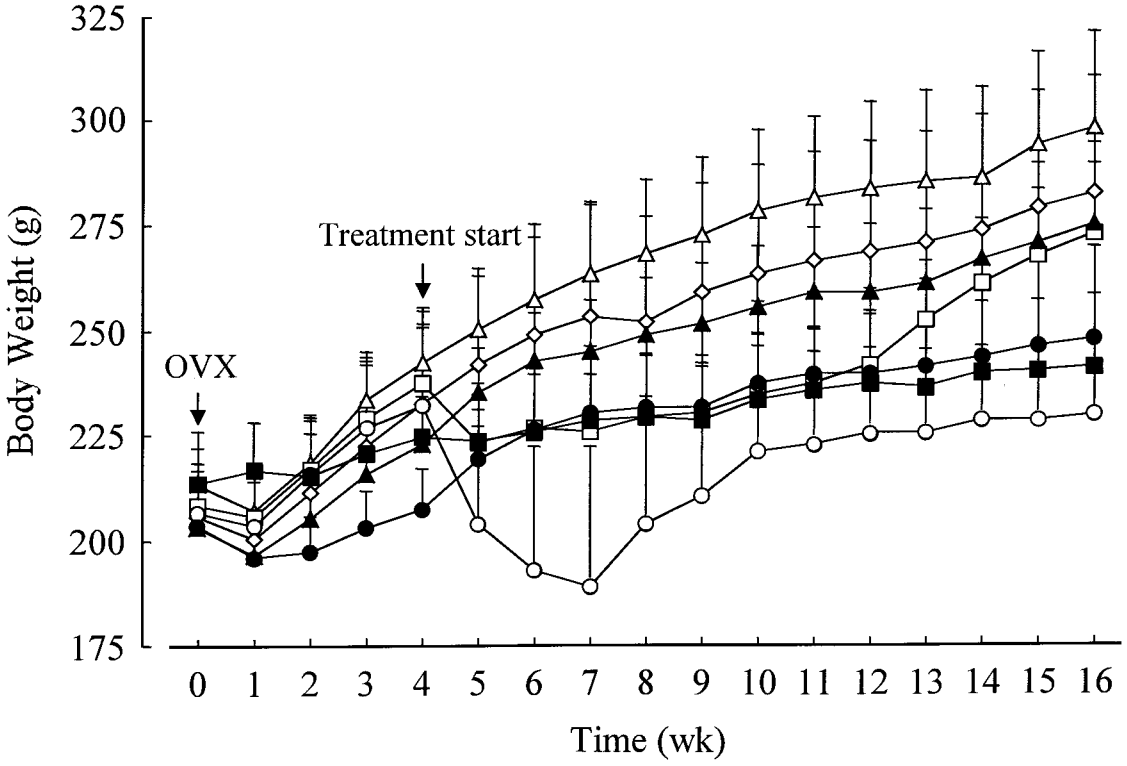

Fig. 3 Changes in body weight during the experimental period.
Values are means ± SD, n = 12 - 15. ■: Basal, ●: Sham, ▲: OVX, ○: OVX + E3, □: OVX + CT, △: OVX + etidronate and ◇: OVX + Ge-132.

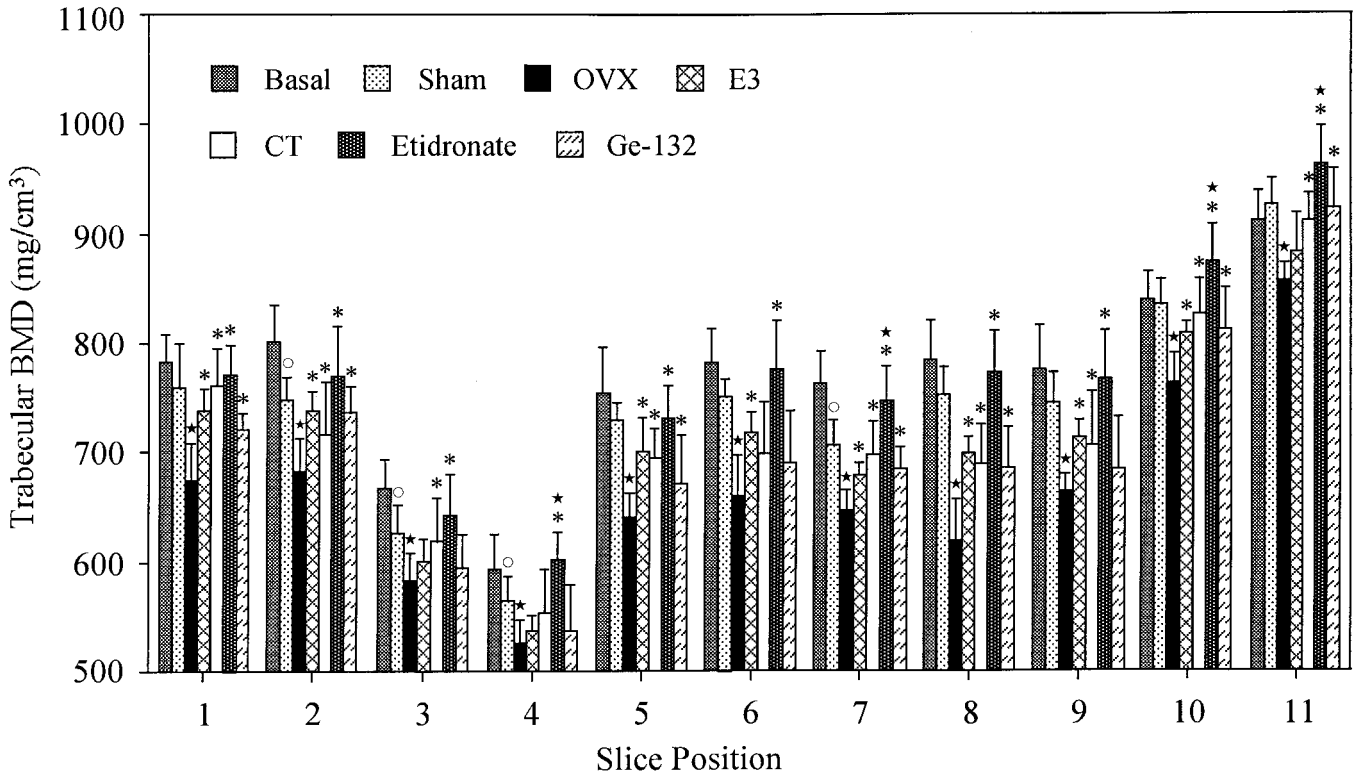

Fig. 4 Changes in trabecular BMD of the mandibular bone.
Data are presented as the mean ± SD, n = 12 - 15. ▩: Basal, ▩: Sham, ■: OVX, ▩: OVX + E3, □: OVX + CT, ▩: OVX + etidronate and ▨: OVX + Ge-132. ∗ $P < 0.05$ (vs. OVX), ★ $P < 0.05$ (vs. Sham), ◯ $P < 0.05$ (vs. Basal).

loss was seen only beneath the first and second molars (slices 8 and 6) in the OVX group than in the Sham group. The therapeutic treatments inhibited the decrease of trabecular BMC in the mandible after OVX. This inhibitory effect on the decrease of trabecular BMC differed among the drugs used. Estriol increased the trabecular BMC of the mandible. In particular, a substantial increase was observed at slices 5, 6 and 8, corresponding to the areas beneath the second and first molars. A similar effect on the trabecular BMC of the mandible was also observed in the Ge-132-treated group. Etidronate and calcitonin treatments substantially increased the trabecular BMC of the mandible. The trabecular BMC of the mandible was greater in the etidronate and calcitonin groups than in the Sham group, showing better protection against bone resorption.

Discussion

Body Weight

Body weight in the OVX group was greater than in the Sham group. This indicates that the increase in body weight was caused by ovary extraction, and that the low-calcium diet (0.1% calcium) had no effect on body weight. Early studies by others have shown a similar increase in body weight after OVX, mainly because of the increase in estrogen deficiency (9,28).

Trabecular Bone Loss in OVX Rats and Effect of Low Calcium

We demonstrated that trabecular BMD and BMC of the mandible were lower in the OVX group than in the Sham group (Figs. 4, 5). This finding indicates that OVX results in trabecular bone loss in the mandible. Decreases in trabecular BMD and BMC in long bones following OVX are well documented in the literature. Trabecular BMD and BMC losses of more than 47% and 50%, respectively, have been reported in lumbar vertebrae of aged osteopenic rats subjected to OVX (11). Such loss is attributable to estrogen deficiency after OVX (11,27). In this study, although BMD and BMC were analyzed in the mandible instead of the lumbar vertebrae, a similar effect of OVX was expected. In addition, the trabecular BMD of the mandible was lower in the Sham group than in the Basal group, indicating that low dietary calcium (0.1% Ca) decreased the trabecular BMD and BMC of the mandible to a greater extent in the Sham group. These results are consistent with those of previous studies in which low dietary calcium induced a significant loss of BMD and BMC in OVX rats (29,30).

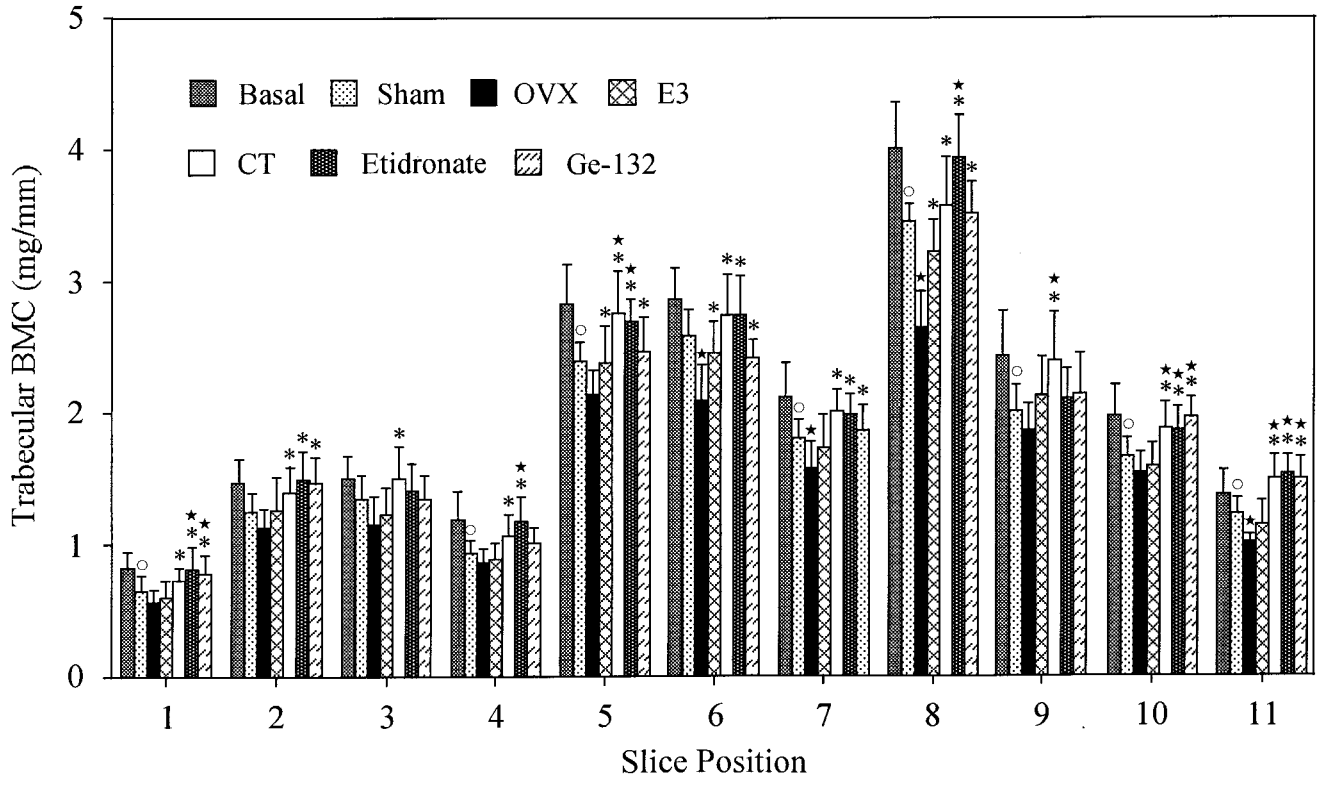

Fig. 5 Changes in trabecular BMC in the mandibular bone.
Data are presented as the mean ± SD, n = 12-15. ▩: Basal, ▩: Sham, ■: OVX, ▩: OVX + E3, □: OVX + CT, ▩: OVX + etidronate and ▨: OVX + Ge-132. ∗ $P < 0.05$ (vs. OVX), ★ $P < 0.05$ (vs. Sham), ◯ $P < 0.05$ (vs. Basal).

Furthermore, the differences in BMD and BMC in the present study were much larger between the OVX and Sham groups than between the Sham and Basal groups. This implies that the degree of BMD and BMC loss due to estrogen deficiency was much greater than that due to low calcium under the present experimental conditions.

Effect of Therapeutic Treatments Against Trabecular Bone Loss

Trabecular BMD and BMC were greater in the therapeutic treatment groups than in the OVX and Sham groups (Figs. 4 and 5). This indicated the effectiveness of the treatment for osteoporosis in OVX rats, although the degree of effect differed among the drugs. There was a significant increase of trabecular BMD and BMC in the etidronate group, indicating that this agent inhibited bone loss in the mandible after OVX. This is consistent with the significantly improved bone mass observed in the alveolar bone and mandibular bone of rats treated with etidronate following OVX (31,32). A study in humans has also shown potential benefits of etidronate therapy for treatment of periodontal disease and periodontitis (33). However, the mechanism of action of this agent has not been precisely defined. Etidronate is one of the bisphosphonate-type compounds that have been adopted relatively recently for treatment of human osteopenia. The cancellous osteopenia induced by OVX is associated with increased bone turnover in terms of indices such as osteoclast surface area, osteoblast surface area and bone formation rate. Hunziker et al. (32) studied the effect of bisphosphonate on mandibular bone formation in aged OVX rats, and noted reduction of bone formation on endosteal surfaces. They concluded that bisphosphonate had a greater anti-resorption effect on endocortical and endosteal surfaces, and that the effect on bone formation was secondary to its effect on bone resorption. Thus, in the present study, the observed potency of etidronate in suppressing bone resorption was thought to be attributable to its marked anti-resorptive effect on endocortical and endosteal surfaces.

The primary action of calcitonin (CT), a polypeptide hormone secreted by the mammalian thyroid gland, is inhibition of bone resorption. Previous investigations (12,13,32,34,35) have reported that CT treatment following OVX prevented bone loss. Shen et al. (35) found that CT treatment after OVX provided complete protection against moderate cancellous bone loss at a skeletal site with a slow rate of cancellous bone loss (the femoral neck) but only partial protection at a skeletal site with a rapid rate of cancellous bone loss (the proximal tibia, where the rate of cancellous bone formation is at least 3 times greater than in the femoral neck). In addition, in a long-term study, Taylor et al. (34) demonstrated the anti-resorptive effect of CT against osteoclast activity and formation. Furthermore, Hunziker et al. (32) reported that CT caused disintegration of mature osteoclasts as well as inhibiting the proliferation of progenitors and the differentiation of precursors to osteoclast cells. In addition, CT caused contraction of the osteoclast cell membrane, an effect possibly related to its inhibition of bone resorption. In the present study, CT increased trabecular BMD and BMC in the mandible of rats (Figs. 4 and 5), the increase in BMC being substantial. However, the observed inhibitory effect of CT on bone resorption in the mandible was weaker than that of etidronate. This is consistent with the findings of Hunziker et al. (32), who confirmed the anti-resorptive effect of CT in the mandible of OVX rats and demonstrated that this effect was weaker than that of bisphosphonate.

It is well known that the net bone loss observed in OVX rats is due to increased bone turnover where bone resorption exceeds bone formation, as a result of estrogen deficiency. The major purpose of estriol therapy is hormone replacement to delay bone loss after the menopause. Previous studies (11,32) have shown that estrogen treatment suppresses bone turnover, resulting in a decreased rate of bone loss and longer maintenance of bone mass. Our results indicated an increase of BMD and BMC after E3 treatment (Figs. 4 and 5), although side effects such as alopecia, body weight loss and poor diet consumption were also seen at the early stage of treatment. Our findings are consistent with those of clinical trials of E3 in postmenopausal women. Nozaki et al. (36) and Minaguchi et al. (37) found an increase of 1.66% and 1.79%, respectively, in the BMD of the spine and a corresponding decrease in biochemical markers after one year of E3 treatment in initially osteopenic women using dual energy X-ray absorptiometry, coupled with an improvement in the Kupperrman menopausal index and good tolerability. Hayashi et al. (38) demonstrated that E3 replacement significantly improved endothelial function and BMD in very elderly women. A similar effect was also reported by Nishibe et al. (39), who established that E3 was effective against senile osteoporosis, and that low-turnover bones were also responsive, in a 10-month study involving 29 women aged 70 - 84 years.

2-Carboxyethylgermanium sesquioxide (Ge-132) is a compound with low toxicity that possesses a wide range of pharmacological effects, such as enhancement of osteoblast activity and prevention of mineral decomposition in senile osteoporosis, in addition to a variety of immunological effects such as modification of biological responses. Previous investigations (22-24) have shown that Ge-132 inhibited bone resorption. Naitou (22) studied

the effect of Ge-132 on the bone resorption activity of neonatal calvaria and the cloned osteoblastic cell line, MC3T3-E1. He found that Ge-132 significantly enhanced ALPase activity and inhibited cAMP production that was stimulated by PTH in MC3T3-E1 cells, and concluded that the inhibitory effect on bone resorption was probably attributable to enhancement of osteoblast activity and inhibition of osteoclast activity. Our previous study (23) in OVX rats showed that the decrease in serum calcitonin (sCT) and increase in serum parathyroid hormone (sPTH) were inhibited by administration of Ge-132, and that the sCT/sPTH ratio was increased. In the present study, Ge-132-treated rats showed increases of BMD and BMC in the mandible (Figs. 4 and 5), suggesting that Ge-132 may also be effective for preventing mandibular bone loss, but may not be as effective as etidronate or calcitonin. Although the mechanism by which Ge-132 counteracts bone loss has not been precisely established, it may occur via direct and indirect action on bone metabolism.

Correlation of Trabecular BMD and BMC with Measured Slice Position

The BMD and BMC in our Basal, Sham, OVX and therapeutic treatment groups varied according to the mandibular slice position at which measurements were taken (Figs. 4 and 5). Similar findings have been obtained in the distal femur and proximal tibia of OVX and Sham rats (40). Furthermore, the BMC was greater in the molar region than at other sites, and BMC was found to decrease from the first molar to the third (Fig. 5). These distinct differences in the trabecular BMD and BMC of the mandible suggested that the osteoporosis had occurred during the experimental period and that the effectiveness of the treatments varied considerably from slice to slice in the mandible. Thus it appears that measurement of BMD and BMC at a single location does not reflect the true degree of osteoporosis in the whole mandible.

It is well accepted that mechanical loading affects bone formation, maintenance and bone mass. Wolff (41) proposed a hypothesis that local formation and resorption of bone occurred in order to allow plasticity of its morphology and internal structure in response to changes in the external physical environment. A later study demonstrated that sensitivity to mechanical stress might differ among bone types (42). Even within the same bone, cells in each location do not show the same responses to dynamic mechanical stress (43). The distribution of occlusal stress in the mandible is also expected to be non-uniform (44), a larger occlusal contact area necessitating a larger bite force. Furthermore, the load transferred by the masticatory muscles affects mandibular bone turnover, and this effect will be dependent on location within the mandible. Our present results showed that the BMC was greater in the molar region than at other sites, and that BMC decreased gradually from the first molar to the third (Fig. 5). This is attributable to the stronger occlusal force on the trabecular bone in the molar region (45), and the gradual increase of maximum bite force and occlusal contact area from the anterior to the posterior molars (44). This effect on bone mass was confirmed by Elovic et al. (46) in a study of OVX rats after tooth extraction. However, in the present study, the maximum BMD was obtained in the mesial area where teeth are absent (Fig. 4). A similar finding has been reported in human mandibular bone (45). Therefore, this high BMD may not be correlated with occlusal stimulus (47). The effect of mechanical stress on bone mass is generally though to be attributable to promotion of osteoblast activity and proliferation, although the details are still unclear. Recent studies have reported that mechanical stress results in production and bursts of activity of signal molecules in bone tissue, such as NO (48), IGF-I and II (49), and COX-2 (50), which are associated with local bone metabolism. Moreover, the differences in trabecular BMD and BMC according to slice position differed among the OVX, Sham and therapeutic treatment groups. This suggested that the changes in bone formation and resorption in the mandible in response to occlusal stress might be correlated with systemic status as well as local occlusal conditions.

In summary, the present study has demonstrated that therapeutic agents can affect trabecular bone loss in the mandible of OVX rats. The therapeutic agents used in this study prevented loss of trabecular bone in the mandible after OVX. Among the agents used, etidronate was the most effective for increasing mandibular BMD and BMC. E3 and Ge-132 treatments also increased mandibular BMD and BMC, Ge-132 treatment being more effective for increasing BMC. CT treatment increased BMC substantially, but had almost no noticeable effect on BMD. The increases in trabecular BMD and BMC in OVX rats due to administration of these agents were found to vary according to location in the mandible.

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