Original Article

The effect of bone morphometric changes on orthodontic tooth movement in an osteoporotic animal model

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ABSTRACT

Objective: To elucidate the effect of bone morphometric changes on orthodontic tooth movement (OTM) in zoledronic acid–treated ovariectomized rats.

Materials and Methods: Twenty-one 10-week-old female Wistar rats were divided into ovariectomy (OVX), OVX with zoledronic acid administration (OVX + ZOL), and sham operation (control) groups. Two weeks after OVX, ZOL administration was initiated. Twelve weeks after OVX, a nickel-titanium closed-coil spring of 25-g force was applied mesially to the maxillary left first molar. In vivo micro–computed tomography (CT) of the left proximal tibia was performed for bone morphometric analysis every 2 weeks after OVX. In addition, OTM was investigated using micro-CT at 0, 12, and 14 weeks after OVX.

Results: There were significant differences in the bone mineral content (BMC), bone volume (BV), BMC to tissue volume ratio (BMC/TV), and BV to TV ratio of trabecular bone between the control and OVX groups and also between the OVX + ZOL and OVX groups. In the OVX + ZOL group, increased BMC and BV in the cortical bone and increased bone mineral density (BMD) in the trabecular bone were observed. Interestingly, OTM in the OVX group was almost two times more than that in the control and OVX + ZOL groups. Moreover, OTM was correlated with BMD, BMC, BV, and BMC/TV in the trabecular bone.

Conclusions: OVX accelerated OTM, while ZOL suppressed it. OTM demonstrated a significant negative relationship with trabecular bone mass. (*Angle Orthod.* 2013;83:766–773.)

KEY WORDS: Zoledronic acid; Tooth movement; Ovariectomy; Micro-CT

INTRODUCTION

With aging of the population, the frequency of aged patients receiving orthodontic treatment has increased recently. Aged patients have many problems associated with physical changes. In particular, changes in bone metabolism are closely related to orthodontic treatment because this entails bone remodeling. Osteoporosis is one of the major diseases characterized by low bone mass and is often associated with aging. In postmenopausal woman, estrogen deficiency is one of the main causes of osteoporosis.

It is important to consider the effects of the drugs used for treatment of osteoporosis on bone metabolism. These drugs include vitamins D and K, calcitonin, selective estrogen receptor modulator, estrogen preparations, and bisphosphonates. Among all of these, bisphosphonates are now one of the optimal choices for treatment of osteoporosis because they strongly inhibit bone metabolic turnover.[1]

Several studies[2–4] reported the effect of bisphosphonates on bone metabolism using micro–computed

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tomography (CT). Borah et al.[5] evaluated changes in bone mineralization and architecture in postmenopausal women with osteoporosis after long-term treatment with risedronate, elucidating normalization of mineral levels. The effect of bisphosphonates on orthodontic tooth movement (OTM) has also been investigated in some reports,[6–10] in which administration of bisphosphonates decreased OTM.

Zoledronic acid (ZOL) is a novel bisphosphonate and is highly effective in preventing bone loss in ovariectomized rats. Perilli et al.[11] reported that treatment with ZOL as late as 2 weeks after ovariectomy (OVX) can still facilitate complete reversal of cancellous bone loss in rat tibia. Sirisoontorn et al.[12] recently reported that OVX accelerated and ZOL inhibited OTM in rats. To date, no study has comprehensively examined OTM and quantitative bone changes in an osteoporotic animal model. Therefore, the present study was conducted to clarify the relationship between bone morphometric changes in the tibia and OTM of the rats in OVX, OVX with ZOL administration (OVX + ZOL), and control groups.

MATERIALS AND METHODS

This study was conducted with approval from the Animal Welfare Committee of Nagasaki University and in accordance with the Animal Experimental Standards of that institution.

Ten-week-old female Wistar rats (SLC, Shizuoka, Japan; body weight, 170–180 g) were used in this study. The rats were housed in plastic cages in a colony room and fed a standard pellet diet and water ad libitum. The rats were allowed 1 week for acclimatization before the experiments began.

Twenty-one rats were randomly divided into the following three groups of seven rats apiece: OVX, OVX + ZOL, and sham operation (control). Bilateral OVX was performed as described previously[13] under general anesthesia by intramuscular injection of ketamine hydrochloride at 87 mg/kg (Ketalar 50; Sankyo, Tokyo, Japan) combined with xylazine hydrochloride at 13 mg/kg (Celactal 2%; Bayer-Japan, Tokyo, Japan). The sham operation was performed in the control group in a manner similar to that used for the other two groups, except for removal of the ovaries. Two weeks after OVX, 3.2 μg/kg ZOL (Zometa; Novartis, Basel, Switzerland) was injected into the peritoneal cavity of the OVX + ZOL group and continued once every 2 weeks (Figure 1A).

After OVX and the sham operation under general anesthesia, 25-g force nickel-titanium closed-coil springs (Sentalloy; Tomy, Fukushima, Japan) were applied to the left maxillary first molar in all groups at 12 weeks to move the molar mesially. Self-cure resin

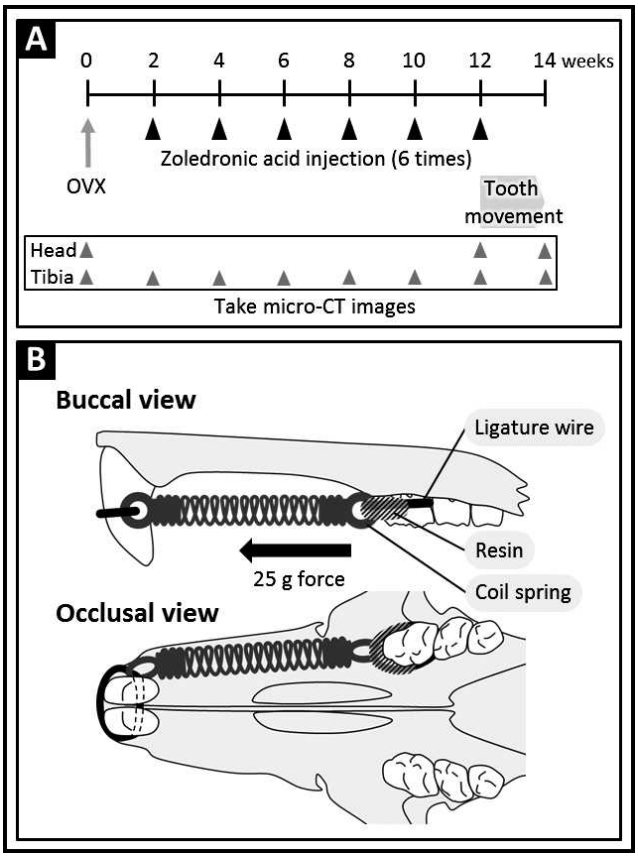

Figure 1. (A) Experimental protocol: 2 weeks after OVX, ZOL administration was initiated in the OVX + ZOL group, and 12 weeks after OVX, experimental OTM was initiated in all of the three groups. (B) OTM appliance design: buccal and occlusal views of the appliance bonded to the mesial aspect of the maxillary left first molars by self-cure resin (diagonal shading).

(Super Bond; Sun Medical, Shiga, Japan) was used to bond the spring on the mesial aspect of the first molar after it was set into place with a 0.008-inch twisted stainless-steel ligature (Figure 1B).

In vivo three-dimensional (3D) micro-CT (RmCT; Rigaku, Tokyo, Japan) images were obtained under general anesthesia. Left proximal tibial images were obtained every 2 weeks after OVX, whereas maxillary left first molar images were obtained at 0, 12, and 14 weeks after OVX. Micro-CT settings were as follows: X-ray source voltage, 90 V; current, 100 μA; scanning time, 2 minutes; and resolution, 20 μm/pixel. OTM was measured by 3D image reconstruction software (i-view; J. Morita, Kyoto, Japan) from the micro-CT images of the same rat with the closed-coil spring at weeks 12 and 14 after OVX. The distance from the most distal contact point of the maxillary left first molar and the most mesial contact point of the maxillary second molar was measured as the amount of OTM. Bone analysis was then performed using 3D medical image analysis software (TRI-BONE; Ratoc

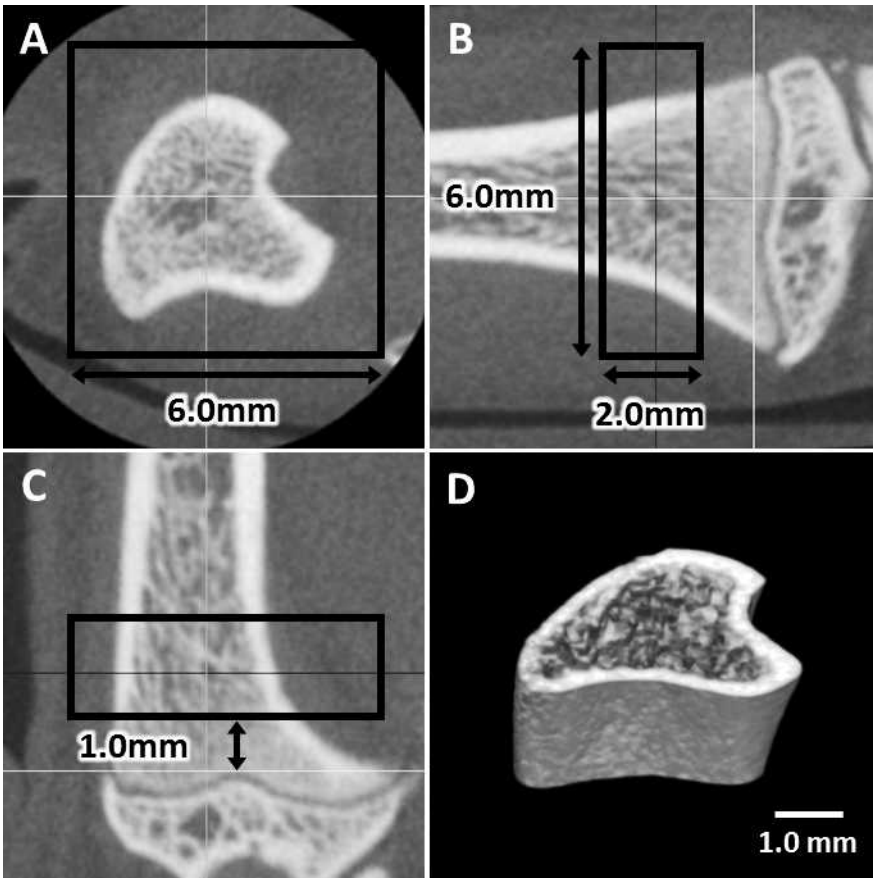

Figure 2. Measurement sites on proximal tibia. The black framed square is the volume of interest (VOI). A: axial section; B: sagittal section; C: coronal section; D: 3D-reconstructed image of the VOI. The VOI was measured 1.0 mm from the distal end of the growth plate and extending 2.0 mm distally.

System Engineering, Tokyo, Japan). A volume of interest (VOI) was created from the left proximal tibial CT image, located 1.0 mm from the distal end of the growth plate and extending distally up to 2.0 mm (Figure 2). The following parameters were calculated for each VOI: bone mineral density (BMD), bone mineral content (BMC), bone volume (BV), trabecular BMC to tissue volume ratio (BMC/TV), and trabecular BV to TV ratio (BV/TV). The same investigator performed all measurements, which were repeated three times. Mean values were used as final measurements.

Statistical Analysis

Statistical analysis was performed using SPSS software (Version 16.0; IBM SPSS, Armonk, NY). The Mann-Whitney *U*-test was used to compare the amount of OTM and the bone morphometric parameters in the three groups. Spearman's rank correlation coefficient was used to evaluate the relationship between the amount of OTM and the bone morphometric parameters.

RESULTS

Orthodontic Tooth Movement

Median OTM was 0.15, 0.20, and 0.10 mm in the control, OVX, and OVX + ZOL groups, respectively (Figure 3). The amount of OTM in the OVX group was two-fold greater than that in the control group and 1.3-fold greater than that in the OVX + ZOL group. Furthermore, OTM in the OVX + ZOL and control groups showed similar results, identical to the findings from our previous study.[12]

Analysis of the Tibia

In the OVX group, significant trabecular bone loss was observed with time. In contrast, accumulation of a dense layer of the trabecular bone close to the growth plate along with hyperplasia of the cortical bone were observed in the OVX + ZOL group (Figure 4).

From week 0 until the end of the experiment, the bone analysis parameters increased gradually, except for BMC, BV, BMC/TV, and BV/TV in the OVX group. The BMD of the cortical bone in the OVX group was

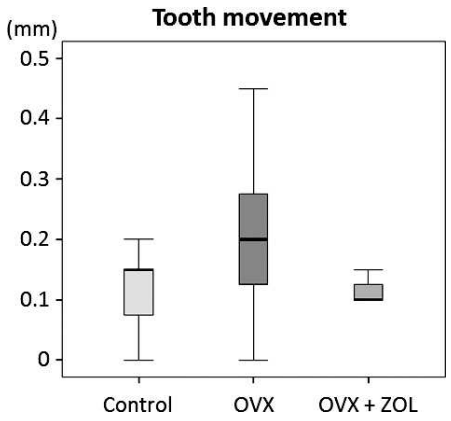

Figure 3. Results of OTM. This figure shows the box plot (ie, box and whisker diagram) of the amount of OTM in the three groups at week 14. The box plot indicates five values of OTM in each group: minimum, first quartile (lowest 25% of data), median, third quartile (highest 25% of data), and maximum.

significantly lower than that in the control group at weeks 4, 6, 10, and 12 (Figure 5A). On the other hand, the BMD of the trabecular bone in the OVX + ZOL group was significantly higher than that in the control and OVX groups from week 10 to week 14 (Figure 5D).

The BMC of the cortical bone in the OVX + ZOL group was significantly higher than that in the control and OVX groups from week 4 to week 14 (Figure 5B). On the other hand, the BMC of the trabecular bone decreased by week 2 in the OVX and OVX + ZOL groups and increased after week 4 in the OVX + ZOL group. The BMC of the trabecular bone in the OVX + ZOL group was almost the same as that in the control group at week 14 (Figure 5E).

The BV of the cortical bone in the OVX + ZOL group was significantly higher than that in the control and OVX groups from week 4 to week 14 (Figure 5C). On the other hand, the BV of the trabecular bone decreased by week 2 in the OVX and OVX + ZOL groups and increased after week 4 in the OVX + ZOL group. The BV of the trabecular bone in the OVX + ZOL group was almost the same as that in the control group at week 14 (Figure 5F).

The BMC/TV and BV/TV were both decreased by week 2 in the OVX and OVX + ZOL groups and increased after week 4 in only the OVX + ZOL group because of the effects of ZOL administration. The BMC/TV and BV/TV in the OVX + ZOL group were almost identical to those in the control group at week 14 (Figure 5G,H).

At week 14, the BMD of the median cortical bone was almost identical in all three experimental groups. The median BMC and BV of the cortical bone in the OVX + ZOL group were almost twofold greater than that in the control and OVX groups. The median BMD of the trabecular bone in the OVX + ZOL group was 1.1-fold greater than that in the control and OVX groups. The median BMC of the trabecular bone in the control and OVX + ZOL groups was 6.9-fold greater than that in the OVX group. The median BV of the trabecular bone in the control and OVX + ZOL groups was demonstrated to measure 5.9-fold greater than that in the OVX group. The median BMC/TV in the control and OVX + ZOL groups was 6.2-fold greater than that in the OVX group. The median BV/TV in the control and OVX + ZOL groups was 5.9-fold greater than that in the OVX group (Table 1).

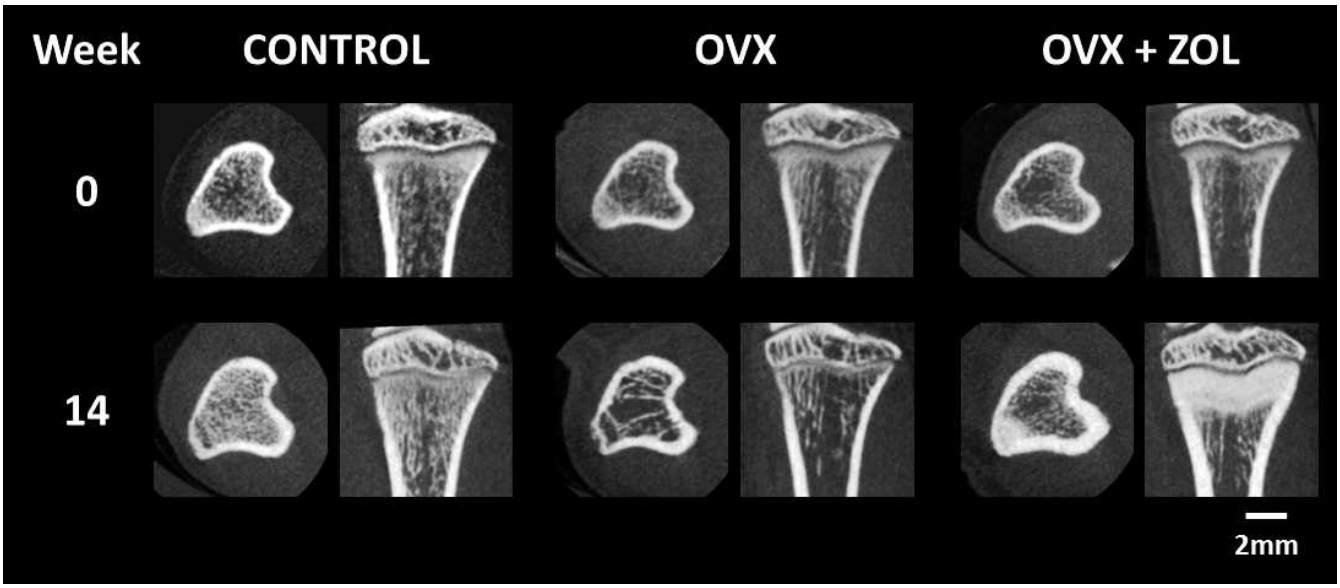

Figure 4. Axial and sagittal section images of tibia in the three groups were obtained in vivo via micro-CT. Images of each group were obtained from the same rat at week 0 and week 14.

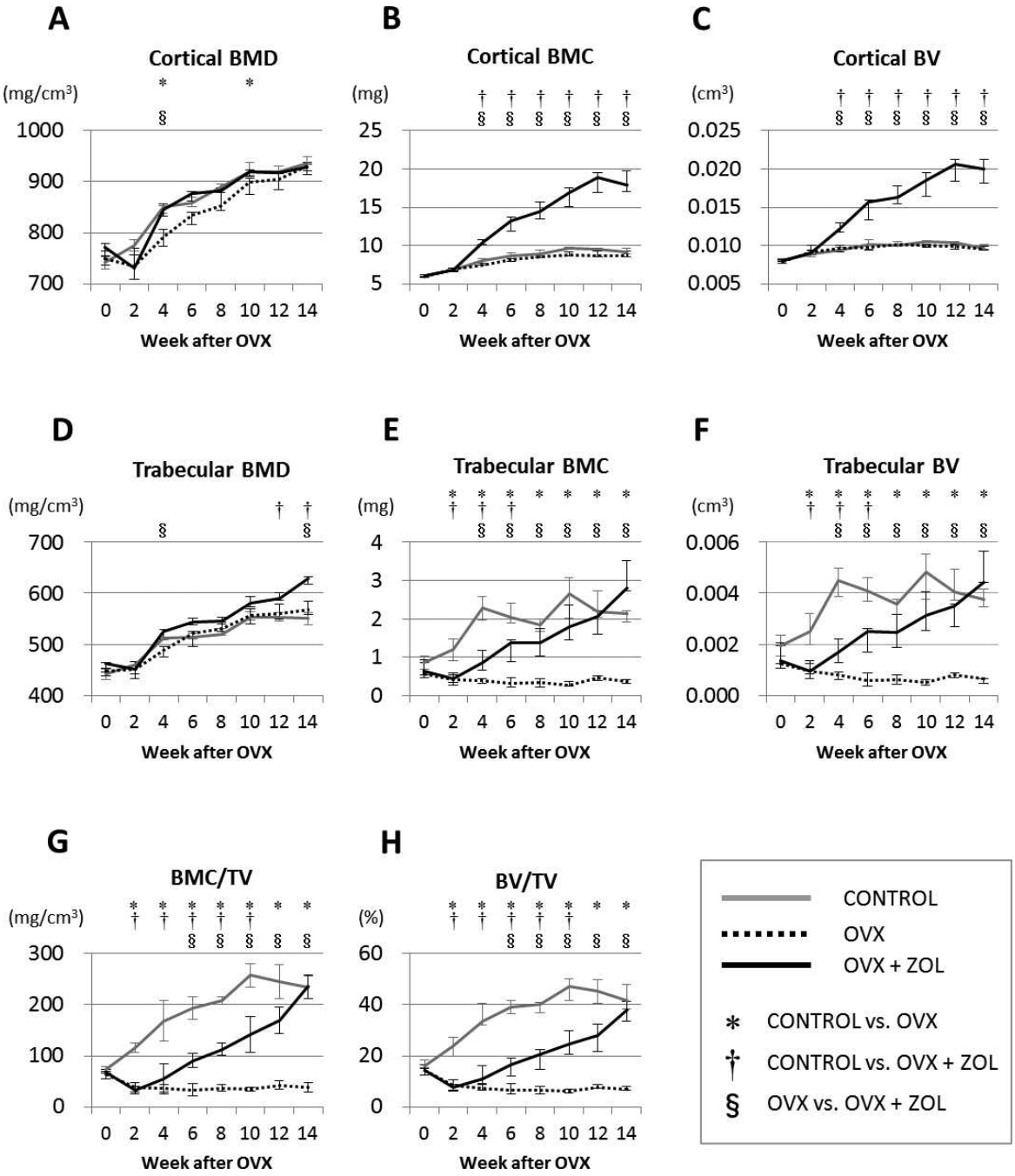

Figure 5. Changes in the bone analysis parameters monitored over time for the three groups. These graphs show medians and interquintile ranges for the entire experimental period. A: cortical BMD; B: cortical BMC; C: cortical BV; D: trabecular BMD; E: trabecular BMC; F: trabecular BV; G: BMC/TV; H: BV/TV. Gray, black broken, and black solid lines represent control, OVX, and OVX + ZOL groups, respectively. * $P < .01$ refers to comparisons between the OVX and control groups; † $P < .01$, the OVX + ZOL and control groups; § $P < .01$, OVX and OVX + ZOL groups (Mann-Whitney *U*-test).

Correlations Between OTM and the Bone Morphometric Parameters

There were significant correlations between OTM and the BMD, BMC, BV, and BMC/TV of the trabecular bone (Spearman's rank correlation coefficients [ρ] were -0.451, -0.492, -0.466, and -0.453, respectively). These values ($0.4 < \rho < 0.7$) indicate moderate correlations. The amount of OTM tended to increase with a decrease in the BMD, BMC, BV, and BMC/TV of the trabecular bone. Nevertheless, there were no significant correlations between OTM and the other bone morphometric parameters (Figure 6; Table 2).

Table 1. Comparison of Orthodontic Tooth Movement (OTM) and Bone Analysis Parameters in Three Groups at Week 14[a]

Group	OTM, mm	Cortical BMD, mg/cm^3	Cortical BMC, mg	Cortical BV, cm^3	Trabecular BMD, mg/cm^3	Trabecular BMC, mg	Trabecular BV, cm^3	BMC/TV, mg/cm^3	BV/TV, %
Control									
Median	0.15	936.93	9.17	0.0097	551.50	2.14	0.0038	234.20	41.60
IQR	0.08	15.10	0.76	0.0007	24.60	0.31	0.0007	45.40	9.80
OVX									
Median	0.20	929.73	8.68	0.0095	567.63	0.36	0.0007	37.90	6.73
IQR	0.15	24.90	0.64	0.0005	26.30	0.08	0.0002	11.60	1.50
OVX + ZOL									
Median	0.10	928.30	17.94	0.0201	629.37	2.80	0.0044	235.67	38.00
IQR	0.03	11.60	2.71	0.0031	14.40	0.79	0.0012	46.50	7.60
P value									
Control vs OVX	0.193	0.180	0.180	0.405	0.110	0.002**	0.002**	0.002**	0.002**
Control vs OVX + ZOL	0.683	0.048*	0.002**	0.002**	0.002**	0.110	0.108	0.949	0.277
OVX vs OVX + ZOL	0.108	0.949	0.002**	0.002**	0.002**	0.002**	0.002**	0.002**	0.002**

[a] BMD indicates bone mineral density; BMC, bone mineral content; BV, bone volume; TV, tissue volume; IQR, interquartile range; OVX, ovariectomy; ZOL, zoledronic acid; and OVX + ZOL, OVX with ZOL administration; * $P < .05$; ** $P < .01$.

DISCUSSION

Bone metabolism is affected by hormones such as parathormone, calcitonin, growth hormone, estrogen, and glucocorticoid. Lack of estrogen, as a result of menopause, accelerates bone resorption, which may lead to osteoporosis-like symptoms. An ovariectomized rat model is widely used to mimic postmenopausal human osteoporosis, with significant decreases in the bone analysis parameters being elucidated in some studies.[2,4] Breen et al.[14] reported that the mean BMD of the trabecular bone in rats 14 weeks after OVX was 19% lower than that in sham rats. However, it was not remarkable in this study (Figure 5D). Other studies[15,16] have reported that the effects of OVX vary depending on the age of the rats. Since all rats in the present study were 10 weeks old (ie, the growth period), the BMD changes after OVX may not have appeared because effects of growth hormone predominate over those of estrogen with regard to the bone metabolism.

In the OVX group, the amount of OTM was maximal among all of the three groups (Figure 3). At the same time, the BMC and BV of the trabecular bone in tibia significantly decreased (Figure 5E,F). Similar changes likely occurred in the alveolar bone, resulting in a greater amount of OTM in the OVX group than in the other two experimental groups. Acceleration of OTM is expedient for orthodontists because it can reduce treatment duration. However, acceleration of TM after OVX is unusual and involves the risk of side effects. Our previous study[13] investigated the effects of OVX on OTM and orthodontically induced root resorption in rats. The results indicated that OVX accelerates OTM but also induces severe root resorption. Therefore, an orthodontist may need to know that osteoporotic patients are well controlled by appropriate administration of medication during orthodontic treatment.

In this study, atypical OTM that was accelerated by OVX recovered to its normal level with ZOL administration (Figure 3). Similar results were observed in the previous study.[12] On the other hand, the BMC and BV of the trabecular bone that were lowered by OVX gradually increased to reach normal levels in the OVX + ZOL group (Figure 5E,F). Furthermore, in this study, the amount of OTM correlated with the trabecular bone parameters of tibia. Therefore, the risk of atypical OTM and/or the amount of OTM may be predicted by identification of bone morphometry in either the tibia or other bones. Some studies[17–20] have indicated that systemic administration of ZOL increased bone parameters not only in the long bones but also in the alveolar bone. In this study, the parameters of cortical bone such as BMC and BV were not affected by OVX although the OTM was increased. On the other hand, these bone parameters were markedly increased as a result of ZOL administration although the OTM was similar to that of control. These results may have caused the insignificant correlation between the parameters of cortical bone and OTM.

With the increasing demand for orthodontic treatment in adult patients, it is necessary for orthodontists to acquire up-to-date knowledge about age-related metabolic changes and effects of medications. Although results from experiments in rat models cannot be extrapolated directly to the human patient, they may offer some guidance.

CONCLUSIONS

- OTM was accelerated in the rats with trabecular bone loss caused by OVX. ZOL suppressed OTM in OVX rats as well as the trabecular bone loss.

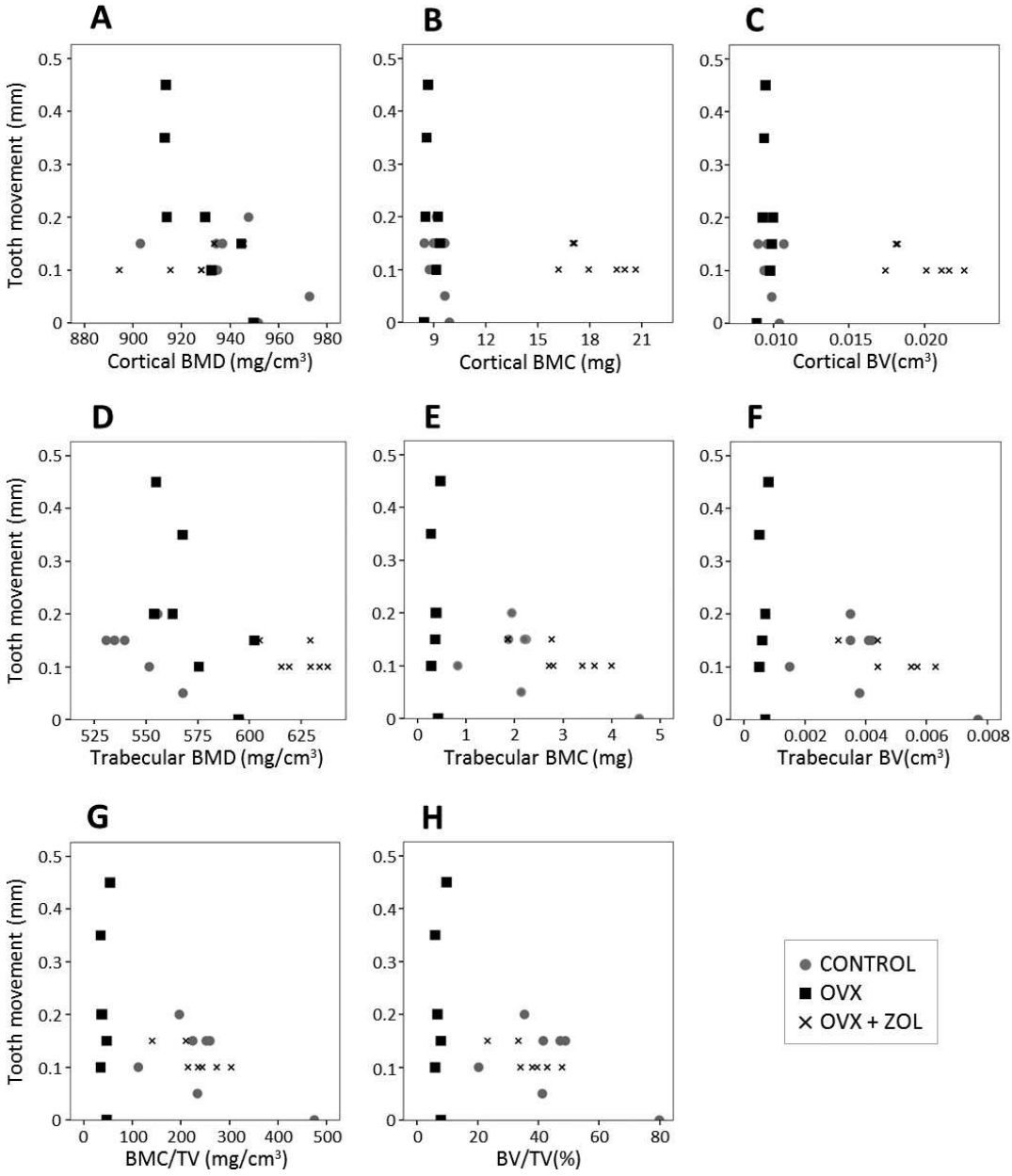

Figure 6. Correlations between the amount of OTM and the bone analysis parameters. These scatter diagrams show the relationship between the amount of OTM and bone analysis parameters. A, cortical BMD; B, cortical BMC; C, cortical BV; D, trabecular BMD; E, trabecular BMC; F, trabecular BV; G, BMC/TV; and H, BV/TV. Circles, squares, and crosses represent the control, OVX, and OVX + ZOL groups, respectively.

Table 2. Spearman's Rank Correlation Coefficient of Orthodontic Tooth Movement (OTM) and Bone Analysis Parameters in Three Groups at Week 14[a]

	Cortical BMD	Cortical BMC	Cortical BV	Trabecular BMD	Trabecular BMC	Trabecular BV	BMC/TV	BV/TV
Correlation coefficient	−0.386	−0.365	−0.289	−0.451	−0.492	−0.466	−0.453	−0.384
P value	.084	.104	.204	.040*	.023*	.033*	.039*	.085

[a] BMD indicates bone mineral density; BMC, bone mineral content; BV, bone volume; and TV, tissue volume; * $P < .05$.

- The negative correlation between OTM and trabecular bone mass of tibia in the rats was found to be statistically significant.

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