Orthodontic tooth movement and root resorption in ovariectomized rats treated by systemic administration of zoledronic acid

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ABSTRACT

Introduction: The effect of zoledronic acid (ZOL), a potent and novel bisphosphonate, on tooth movement and orthodontically induced root resorption (OIRR) in osteoporotic animal model systemically treated with ZOL similarly used in postmenopausal patients has not been elucidated. Therefore, the present study was investigated. **Methods:** Fifteen 10-week-old female Wistar rats were divided into three groups including OVX (ovariectomy), OVX+ZOL and control groups. Only OVX and OVX+ZOL groups had undergone ovariectomy. Two weeks after OVX, ZOL was administered into only OVX+ZOL group. Four weeks after OVX, 25-g nickel-titanium (NiTi) closed-coil springs were applied to observe tooth movement and OIRR. **Results:** There were significant differences in the amount of tooth movement and OIRR between OVX and control groups also between OVX and OVX+ZOL groups. There was no statistically significant difference in the amount of tooth movement and OIRR between OVX+ZOL and control groups. ZOL inhibited significantly more tooth movement and significantly reduced the severity of OIRR observed in ovariectomized rats. OVX+ZOL group showed almost the same results as the control group in both tooth movement and OIRR. **Conclusions:** ZOL inhibits the excessive amount of orthodontic tooth movement and also reduces the risk of severe OIRR in ovariectomized rats.

INTRODUCTION

Several reviews published over the years have focused on the effects of systemic or local application of several drugs during orthodontic tooth movement.[1-3] Bisphosphonates have been the most common prescribed medications for osteoporotic patients[4-6] and their effects on orthodontic tooth movement have been investigated in rat model.[7-10] Essentially, bisphosphonates are internalized into osteoclasts leading to inhibition of bone resorption and induction of osteoclast apoptosis.[11-13]

ZOL is a potent and novel bisphosphonate that has been recently shown to significantly reduce fracture risk in the patients who received once-yearly dosing regimen for the treatment of postmenopausal osteoporosis.[14] According to several studies,[4-6] ZOL could be considered to be the most potent inhibitor of bone resorption comparing with other bisphosphonates. ZOL is a nitrogen-containing, third-generation bisphosphonate (N-BPs) which has a mechanism of action different from that of the nonnitrogen-containing bisphosphonates. A recent study has elucidated the pharmacological properties of N-BPs resulting in the induction of osteoclast apoptosis.[15] Moreover, ZOL has been advocated

by several histological and micro-CT studies showing highly effective prevention of bone loss in ovariectomized rats.[16-20]

Rat and mouse models have been commonly used for the studies of orthodontic tooth movement and OIRR. Sirisoontorn et al.[21] has reported that orthodontic tooth movement in the ovariectomized rats has been more rapid than the control rats and OIRR in the ovariectomized rats was more severe than the control rats. A recent study by Fujimura et al.[22] has been reported that the local administration of alendronate sodium hydrate has inhibited orthodontic tooth movement and root resorption in male mice. Classically, ovariectomized rats have been advocated as a good osteoporotic animal model[23] and often have been used to study efficacy of drugs aimed to prevent bone loss.[16,24-28]

Due to the similarity among of the morphology and function of the cells, in both, root resorption and bone resorption processes could be considered similar.[29] In addition, root resorption has been involved not only in osteoclastogenesis but also in odontoclastogenesis via the OPG/RANK/RANKL system.[30] Therefore, the medications and other substances have been used to inhibit the bone resorption such as bisphosphonates might show an effect on the prevention of root resorption during orthodontic treatment.[22]

In the last 20 years, the number of adult orthodontic patients has dramatically increased.[31] However, the studies about pharmaceutics-orthodontics in adult orthodontic patients, especially in postmenopausal patients, have never been completely elucidated due to the continually developing drugs and drug regimens.

To date, to our knowledge, there is no study reporting on the relationship between orthodontic tooth movement and OIRR in the ovariectomized rats using systemic administration of ZOL. Therefore, the aim of the present study was to investigate the effect of ZOL at the same administration as used in postmenopausal patients following orthodontic force application in an osteoporotic rat model.

METHODS

This study was conducted with the approval from the Animal Welfare Committee of Nagasaki University. Some parts of this study were modified following the previous study.[21] Fifteen 10-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*. After arrival, the rats were allowed one week for acclimatization prior to the

commencement of the experiments. Fifteen rats were randomly divided into three groups of five each: OVX and OVX+ZOL experimental groups and control group.

Bilateral OVX was performed under general anesthesia by intramuscular injection of ketamine hydrochloride at a dose of 87 mg/kg (Ketalar 50, Sankyo, Tokyo, Japan) combined with xylazine hydrochloride at a dose of 13 mg/kg (Celactal 2%, Bayer-Japan, Tokyo, Japan).[21,32] Briefly, the surgical procedure comprised: (1) hair removal over the surgical areas, (2) small incisions (~1.0 cm) were made into the skin halfway between the middle of the back and the base of the tail on both left and right sides, (3) whole ovary removal with a scalpel inserted between the fallopian tubes and the uterine horns, (4) surgical skin closure.[33] Sham operations were also performed in the control group, in which all of the other procedures were exactly the same, except for the removal of the ovaries. Two weeks after OVX, ZOL (Zometa, Novartis, Basel, Switzerland) was injected into the peritoneal cavity (1.6 μg/kg) of the OVX+ZOL group[34], and continued once a week totally 6 times. The last dose was injected on the same day coinciding with day 21 of the experimental orthodontic tooth movement (Fig 1 A).

Experimental Orthodontic Tooth Movement

Four weeks after OVX or sham operations, all experimental groups were applied 25-g nickel-titanium (NiTi) closed-coil springs (Sentalloy, Tomy, Fukushima, Japan) under general anesthesia as previously described[21] to move the maxillary left first molars mesially. The maxillary right first molars without any orthodontic force application served as the negative controls. After the closed-coil springs were set, a self-cured resin (Super Bond, Sun Medical, Shiga, Japan) was applied from the disto-buccal line angle to the disto-palatal line angle on the twisted 0.008” ligature wires which were fixed around the maxillary left first molars (Fig 1 B-D).

In vivo 3D micro-CT (RmCT, Rigaku, Tokyo, Japan) was taken under general anesthesia as previously described[21] at day 0 (before and immediately after the closed-coil springs were applied), 1, 3, 7, 14, 21, and 28 (before and immediately after the closed-coil springs were removed). At day 28, all rats were sacrificed by CO_2 inhalation. Orthodontic tooth movement 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 day 0 and day 28. The distance from the most distal contact point of the maxillary left first

molars and the most mesial contact point of the maxillary second molars was calculated to represent the amount of orthodontic tooth movement between groups. Furthermore, the micro-CT images from the same rat without the closed-coil spring at day 0 and day 28 were used as the representative images from different groups for superimposition purposes to analyze tooth movement by using 3D medical image analysis software (Ratoc, Ratoc System Engineering, Tokyo, Japan). The same investigator performed all measurements and every measurement was repeated at three separate times. The mean value was used as the final measurement.

Orthodontically Induced Root Resorption

After the experimental orthodontic tooth movement was quantified, the rats were sacrificed. The experimental and negative control molars were extracted. All extracted first molars were submerged in 1% sodium hypochlorite for 10 minutes to eliminate the periodontal ligament remnants. The five roots of the first molars were divided into three parts using the diamond discs (Fig 1 E). Only the mesial and the distal roots (disto-buccal and disto-palatal) were used in this study (Fig 1 F). The mesial surfaces of the roots were scanned using SEM (TM-1000, Hitachi, Tokyo, Japan). The areas of the resorption craters were measured by 3D

medical image processing software (Mimics, Materialise, Leuven, Belgium), and the depths of the root resorption craters were evaluated with 3D laser scanning microscope (VK-8500, Keyence, Kyoto, Japan) following the image analysis software (Scion Image, Scion, Maryland, USA). The same investigator performed all measurements and all measurements were repeated at three separate times. The mean value was used as the final measurement.

Statistical Analysis

Statistical analysis was performed using SPSS software (SPSS version 16.0, IBM, New York, USA). The Mann-Whitney test was used to compare the amounts of orthodontic tooth movement and OIRR between the experimental groups.

RESULTS

Experimental orthodontic tooth movement was quantified using 3D micro-CT images at different experimental days (Fig 2, Fig 3 A). The amounts of orthodontic tooth movement gradually increased from day 0 until the end of the experiments in all groups. The amount of orthodontic tooth movement in the OVX group was obviously greater than the OVX+ZOL group and the control group at all time points of the

experiments (Fig 3B, Table I). Furthermore, the amount of orthodontic tooth movement in the OVX+ZOL group and the control group showed similar results during all experimental time points (Fig 2, Fig 3, Table I). A graph of orthodontic tooth movement between the OVX+ZOL group and the control group (Fig 3 B) clearly indicate an almost complete overlap with each other during the experiments.

The superimposition images from the sagittal, the axial and the coronal views are shown to represent the amount and direction of orthodontic tooth movement in all experimental groups. The differences at day 0 (blue) and day 28 (red) are observed by the overlapped areas of micro-CT images showing in purple (Fig 3, A). Moreover, the micro-CT images of all experimental groups at day 28 were used to compare the amount and the direction of orthodontic tooth movement. The results of orthodontic tooth movement in the OVX+ZOL group show almost the same distance as the control group evidenced by an almost complete overlap with each other in all sectional views. However, the results of the OVX group are only partial overlapped by the OVX+ZOL group and the control group in all sectional views (Fig 3A).

Small amounts of initial orthodontic tooth movement were found in all experimental groups at day 1. These median values were 0.05 mm in

the OVX+ZOL group and in the control group. The greatest amount of the initial orthodontic tooth movement was found 0.07 mm in the OVX group (Fig 3B, Table I). Dramatic increases of orthodontic tooth movement were found from day 7 until the end of the experiments in all groups, especially in the OVX group (Fig 3B, Table I). At day 28, the amounts of orthodontic tooth movement were found 0.36 mm in the OVX+ZOL group and 0.35 mm in the control group. The greatest amount of orthodontic tooth movement at day 28 was found 0.72 mm in the OVX group. There were significant differences in the distance of orthodontic tooth movement between the OVX group and the control group ($P < 0.01$, at day 7 and 14; $P < 0.05$ at day 1, 3, 21 and 28) (Fig 3, B; Table I). In addition, there were significant differences in the distance of orthodontic tooth movement between the OVX group and the OVX+ZOL group ($P < 0.05$ at day 1, 3, 7, 14, 21 and 28) (Fig 3B, Table I). Interestingly, there was no significant difference in the distance of orthodontic tooth movement between the OVX+ZOL group and the control group during the experimental time points (Fig 3B, Table I).

The results of OIRR are shown using SEM images (Fig 4). Three types of OIRR were clearly identified in all experimental groups: isolated lacunae, wide and shallow resorption pits, and deep resorption craters.

Small isolated lacunae were mainly seen on the cervical half of the mesial surfaces on the mesial roots in the OVX+ZOL group and the control group. Wide craters were mainly seen on the disto-buccal and the disto-palatal roots covering the cervical and the middle portions of the roots in the OVX+ZOL group and the control group. In contrast, wide and deep resorption craters were observed in the OVX group scattered on the mesial, the disto-buccal and the disto-palatal roots. These resorption craters were not only at the cervical and the middle portions but also at the apical portions of the roots.

The results of OIRR in the OVX group showed certainly more severe root resorption than the OVX+ZOL group and the control group in the area, the depth and the volume of OIRR (Table II) quantified on the mesial, the disto-buccal and the disto-palatal roots. Furthermore, the results of the total volume of root resorption in three roots in the OVX group showed larger root resorption craters than the OVX+ZOL group and the control group (Fig 5, Table III). Almost the same severity of OIRR were found between the OVX+ZOL group and the control group in all the parameters, i.e. the area, the depth and the volume of OIRR (Table II) investigated on the mesial, the disto-buccal and the disto-palatal roots. Moreover, the results of the total volume in three roots of

the OVX+ZOL group showed almost the same severity as in the control group (Fig 5, Table III). There were significant differences ($P < 0.05$) in the area, the depth, and the volume of OIRR between the OVX group and the control group analyzed in the mesial, the disto-buccal and the disto-palatal roots also the total volume of OIRR in three roots (Fig 5). Similarly, there were significant differences ($P < 0.05$) in the severity of OIRR between the OVX group and the OVX+ZOL group in the area, the depth and the volume of OIRR identified on the mesial, the disto-buccal and the disto-palatal roots also the total volume of OIRR in three roots (Fig 5). However, there was no significant difference in the severity of OIRR between the OVX+ZOL group and the control group in the area, the depth, and the volume of OIRR indicated on the mesial, the disto-buccal and the disto-palatal roots also the total volume of OIRR in three roots (Fig 5).

DISCUSSION

We previously investigated the effects of OVX on orthodontic tooth movement and OIRR during the experimental orthodontic force application in the rats.[21] Our previous study showed identical results with the present study in both orthodontic tooth movement and OIRR.

Additionally, the present study also revealed the initial orthodontic tooth movement at day 1 in all experimental groups. There were significant differences between the control group and the OVX group also between the OVX group and the OVX+ZOL group in initial orthodontic tooth movement at day 1 (Table I). These small amounts of orthodontic tooth movement might be resulted from the initial compressions within the periodontal ligament spaces after orthodontic force applications.[21] In addition, the OVX group showed the greater distance of initial orthodontic tooth movement than the OVX+ZOL and control groups because the ovariectomized rats have been indicated to reduce the stiffness and elastic modulus of alveolar bone resulting in severe microarchitectural change.[35] Besides, the present study also found a gradually increase of orthodontic tooth movement from day 0 until day 7. After day 7, orthodontic tooth movement showed an ascending slope rising up until day 21. The maximal increase was observed between day 21 and day 28 in all experimental groups (Fig 3B, Table I). Additionally, the OVX group showed the greatest distance of orthodontic tooth movement at all experimental time points. Similarly, previous studies that evaluated the effects of systemic-osteoporotic hormonal imbalance on orthodontic tooth movement showing the increased rate of orthodontic tooth movement in the osteoporotic alveolar bone due to

the effects of OVX have influenced on the increase of the bone turnover resulting in the acceleration of orthodontic tooth movement.[36,37]

Furthermore, the OVX was reported to increase the indices of the bone resorption in the rat tibia at 2 weeks after OVX, and the maximal increase in the bone resorption parameters consequently occurred in the few months.[38] The theories of root resorption have been clarified via several molecular and cellular mechanisms to exemplify the pathways of root resorption. The OPG/RANK/RANKL system has been described as an important role in osteoclastogenesis and odontoclastogenesis via the balance between OPG and RANKL on the tension and the compression side of the tooth during orthodontic tooth movement[30] also has been modulated by the loss of estrogen such as OVX resulting in the increase of osteoclastogenesis.[39] Therefore, the effects of OVX have played a role in the bone turnover mechanism arising from the reduction of the estrogen levels in the OVX group.[40,41] Due to the similarity between the morphology and function of the cells, root resorption and bone resorption processes could be considered similar.[29] Therefore, hormonal changes involved in bone resorption process might be also involved in root resorption process. Consequently, OIRR in the OVX group of the present study was remarkably more severe than in the OVX+ZOL group and the control group (Table II; Table III).

Additionally, the results of OVX+ZOL group in the present study were similar to the control group in both orthodontic tooth movement and OIRR. From several studies,[4-6] ZOL could be considered to be the most potent inhibitor of the bone resorption compared with other bisphosphonates due to the mechanisms of action and pharmacological properties directly involving the induction of osteoclast apoptosis.[15] Thus, the injection of ZOL in present study into the OVX+ZOL group might be involved in the decrease of osteoclastogenesis caused by the induction of osteoclast apoptosis. This might be the result in the decrease of orthodontic tooth movement and OIRR in the OVX+ZOL group compared with the OVX group (Table II, Table III). In the present study, we started to inject ZOL by intraperitoneal administration into the OVX+ZOL group after 2 weeks of OVX. This time point has been remarked as the early time point when major degenerative bone changes were found.[28,42,43] Recently, a study has elucidated that after 4 weeks of OVX (only 2 weeks after the injection of ZOL was started), the OVX group has shown the loss of the bone volume fraction in 64 %, whereas the OVX+ZOL group has shown the bone volume fraction similarly to the baseline.[34] In our study, we started to apply orthodontic force 4 weeks after OVX and we found almost the same results of both orthodontic tooth movement and OIRR in OVX+ZOL and control groups.

The main reasons to explain this phenomenon might be the experimental orthodontic tooth movement in the OVX+ZOL group was performed at experimental time point when the bone volume fraction might have returned to be the same as the control group level since ZOL had been already injected twice. This is likely according to the effects of ZOL in the first 2 weeks of the injection mainly preserved and restored the cancellous bone microarchitecture to achieve the microarchitectural structures and patterns similar to the control group.[34]

The results of present study are also in agreement with our previous study[21] in which ovariectomized rats showed an increase in orthodontic tooth movement and OIRR via the bone metabolism pathways resulting from hormonal imbalance. Moreover, the present study might have the important implications for the future osteoporosis studies especially in the experimental orthodontic tooth movement and OIRR because the results of the first 2 weeks post-OVX in the rats could be a reference time point to achieve the effective recovery of the bone volume by injection of ZOL.

From past to present, the roles of bisphosphonates in orthodontic field still have been advocated as the inhibitors on orthodontic tooth movement. However, in our present study, we used ZOL which have never been studied in the ovariectomized rats by systemic administration

similarly used in postmenopausal women. The results from an osteoporotic rat model following orthodontic force application, indicate that ZOL inhibits the excessive amount of orthodontic tooth movement, and also reduces the risk of severe OIRR. Therefore, orthodontists should be aware of while treating the postmenopausal patients without taking any antiresorptive drugs. The medical history taking should be the routine for the patients who require the orthodontic treatment to inform about the drugs. The most important consideration for the postmenopausal patients who have never been treated with bisphosphonates and request orthodontic treatment is to understand the chance that the orthodontic tooth movement could be more excessively rapid also OIRR could be more severe than the premenopausal patients. These reasons may reduce the success of orthodontic treatment. Bisphosphonates may increase the possibility of localized jaw osteonecrosis especially in the bisphosphonate-treated postmenopausal patients requiring surgical procedures (e.g., orthognathic surgery, extractions, periodontal surgery, and implants).[44] Thus, physicians or dentists have become increasingly concerned about the possible risk of this disorder in their patients on long-term bisphosphonate therapy, prompting organizations to issue management guidelines for this disorder.

The results from an animal experiment cannot be directly extrapolated to a clinical situation. However, these results provide new insights in this field.

CONCLUSIONS

ZOL inhibits the excessive amount of orthodontic tooth movement and also reduces the risk of severe OIRR in ovariectomized rats.

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FIGURE CAPTIONS

Figure 1. Orthodontic tooth movement in the ovariectomized rats.

A, Experimental time points of ZOL administration and experimental orthodontic tooth movement. **B and C,** Appliance design in the buccal (day 0) and occlusal (day 28) views, respectively. (R) indicate the area of self-cured resin and (W) indicate the twisted ligature wire. **D,** Intraoral photograph of the appliance *in situ* at day 0. **E and F,** Maxillary right first molar (negative control) with separation lines in the buccal and apical views, respectively. Three roots are labeled including the mesial (M), disto-buccal (DB) and disto-palatal (DP) roots.

Figure 2. 3D micro-CT reconstruction images.

A, 3D micro-CT reconstruction image showing the orthodontic appliance *in situ* at day 0. The arrows show the direction of force application. **B,** Comparison of orthodontic tooth movement in the control, OVX and OVX+ZOL groups at day 0 and 28.

Figure 3. 3D micro-CT superimposition images and a graph of orthodontic tooth movement.

A, 3D micro-CT superimposition images of the control, OVX and OVX+ZOL rats at day 0 (blue) and day 28 (red) are shown in the sagittal, axial and coronal views. The overlapped areas are shown in purple. The arrows show the direction of orthodontic force. The bottom row shows the results at day 28. The control (blue), OVX (green) and OVX+ZOL (red) are shown in the sagittal, axial and coronal views. The overlapped areas are shown in teal, yellow, purple and white. The arrows show the orthodontic force direction. **B,** A graph of orthodontic tooth movement at day 0, 1, 3, 7, 14, 21 and 28 is shown in the control group, OVX and OVX+ZOL groups. The light grey asterisks refer to the comparisons between the OVX and control groups. In addition, the black asterisks refer to the comparisons between the OVX and OVX+ZOL groups.

* $P < .05$, ** $P < .01$ (Mann-Whitney test)

Figure 4. Scanning Electron Microscope (SEM) images.

Mesial (M), disto-buccal (DB) and disto-palatal (DP) roots of the maxillary left first molars are shown. The areas of OIRR are shown in black.

Figure 5. Box plots of total volume of OIRR in three roots.

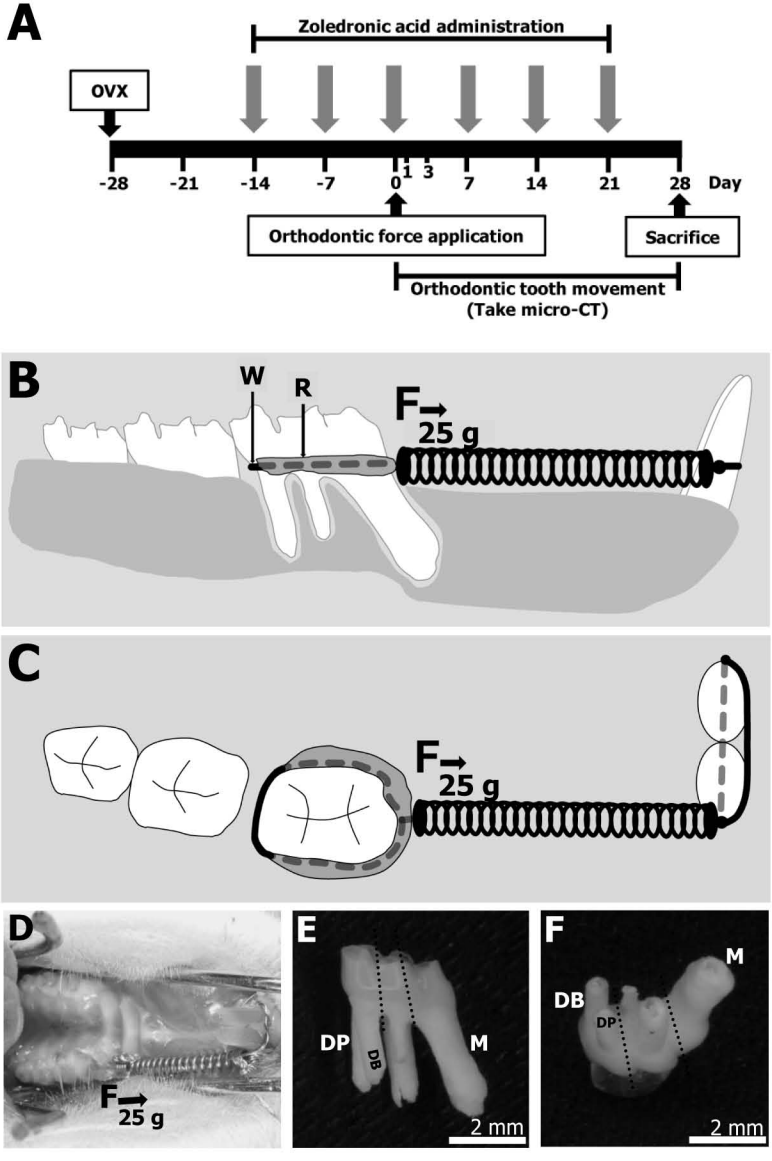
A
Zoledronic acid administration
OVX
-28
-21
-14
-7
0
1
3
7
14
21
28
Day
Orthodontic force application
Sacrifice
Orthodontic tooth movement
(Take micro-CT)
B
W
R
F
25 g
C
F
25 g
D
F
25 g
E
DP
DB
M
2 mm
F
M
DB
DP
2 mm

Figure 1

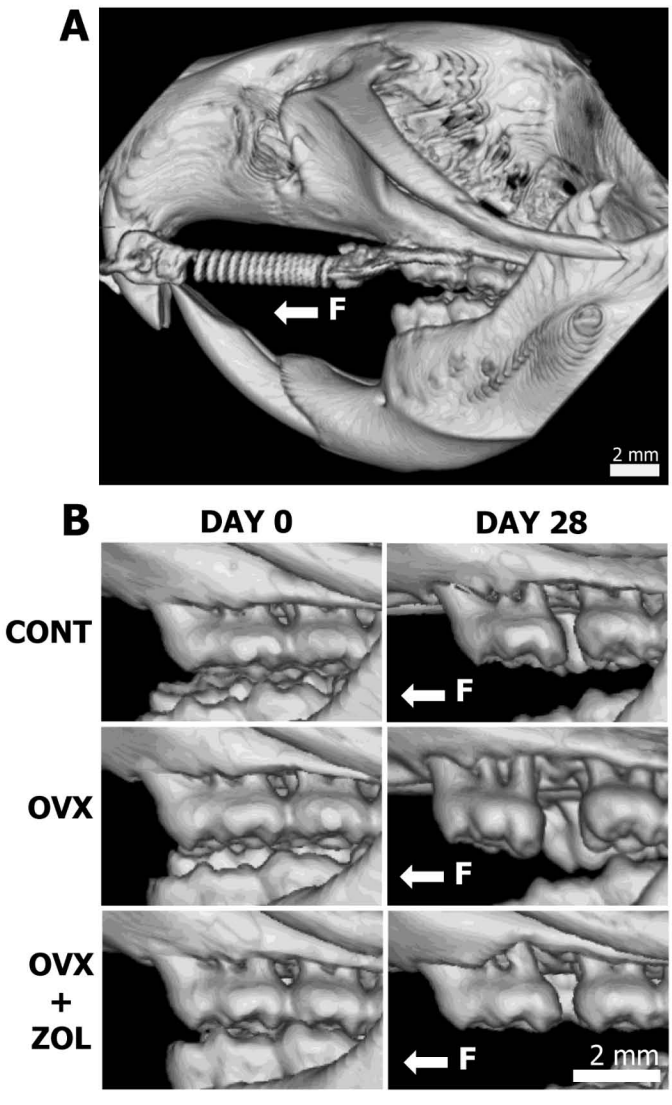

Figure 2

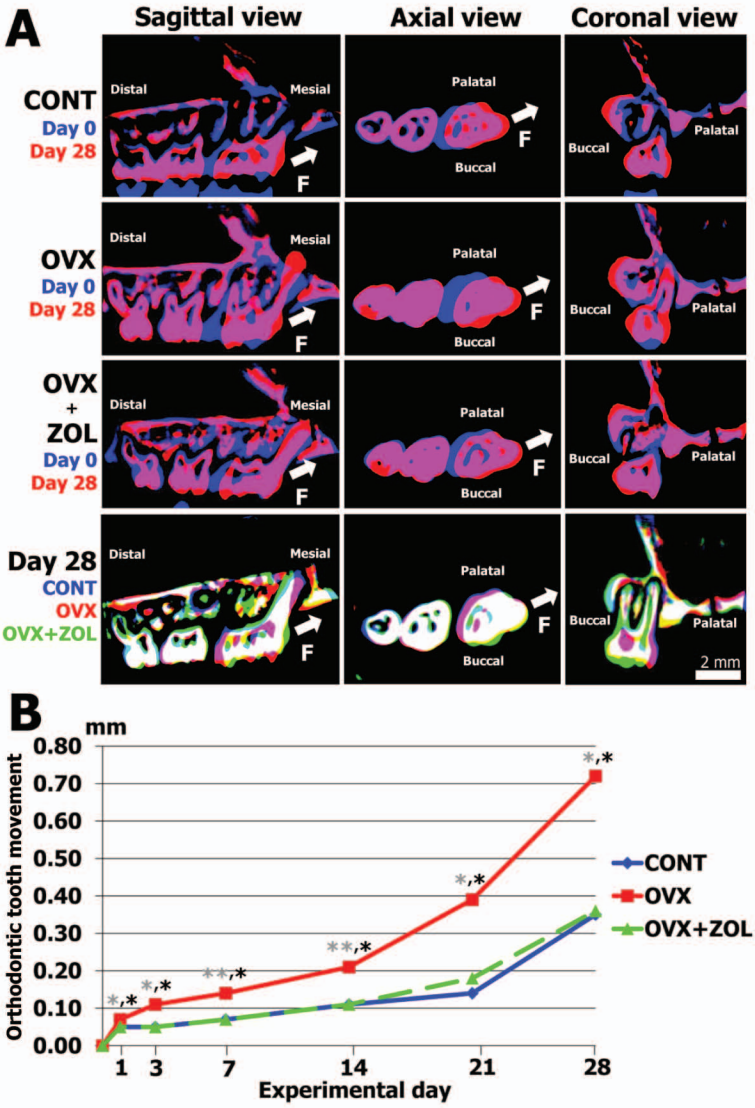
A
Sagittal view
Axial view
Coronal view
CONT
Day 0
Day 28
OVX
Day 0
Day 28
OVX
+
ZOL
Day 0
Day 28
Day 28
CONT
OVX
OVX+ZOL
Distal
Mesial
Palatal
Buccal
F
2 mm
B
mm
Orthodontic tooth movement
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
1
3
7
14
21
28
Experimental day
CONT
OVX
OVX+ZOL

Figure 3

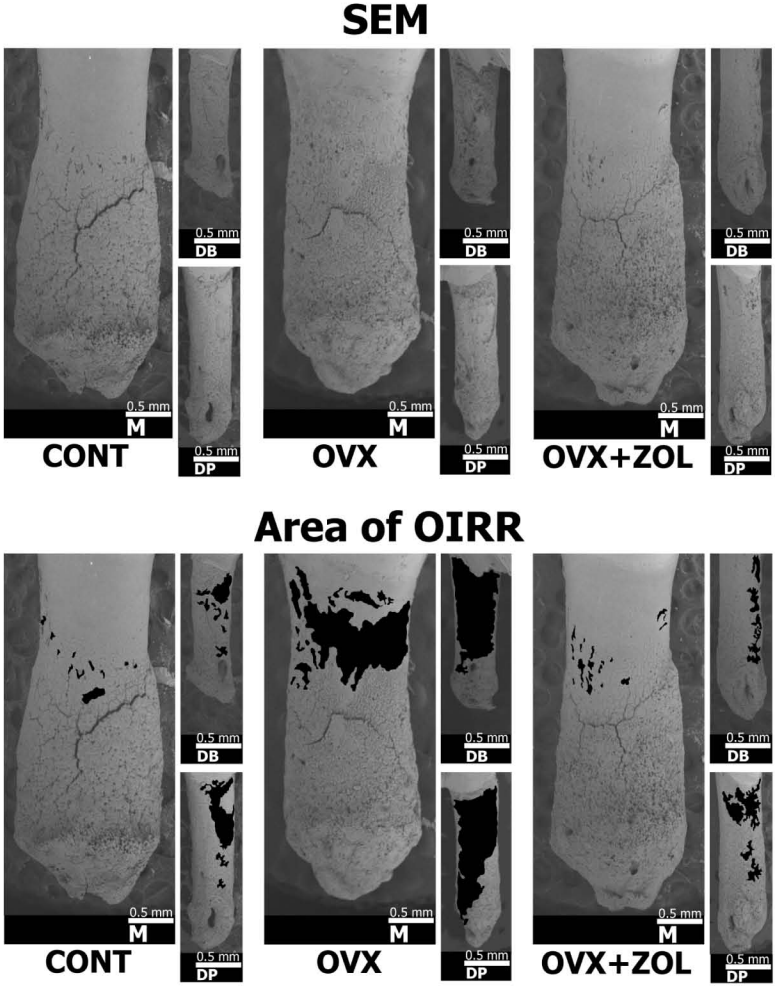

Figure 4

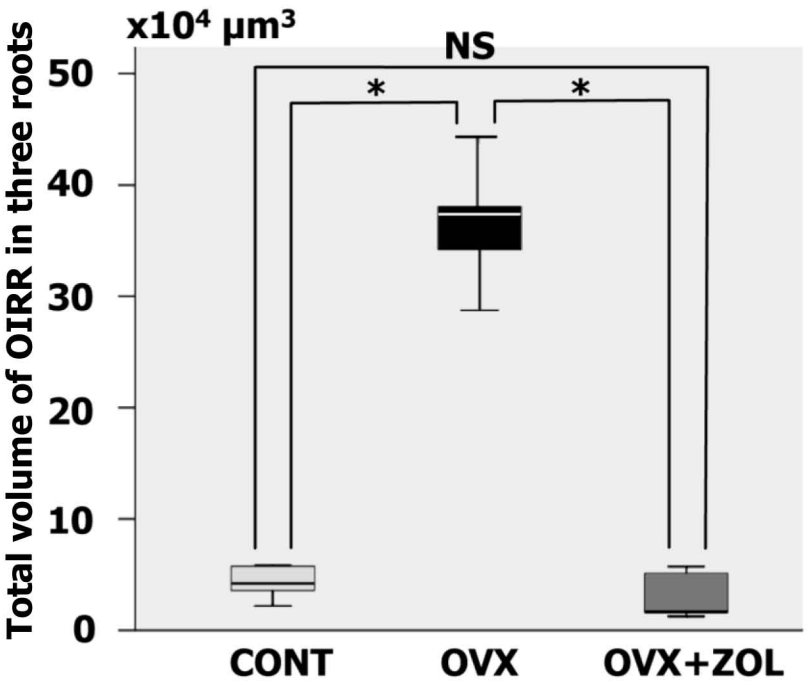
x10⁴ μm³
Total volume of OIRR in three roots
50
40
30
20
10
0
NS
*
*
CONT
OVX
OVX+ZOL

Figure 5

Table 1

Comparison of orthodontic tooth movement between the control, OVX and OVX+ZOL groups at day 0, 1, 3, 7, 14, 21 and 28.

Rats	Tooth movement (mm)						
	CONT						
	D 0	D 1	D 3	D 7	D 14	D 21	D 28
1	0.00	0.05	0.05	0.07	0.14	0.25	0.42
2	0.00	0.05	0.05	0.07	0.11	0.18	0.46
3	0.00	0.00	0.00	0.05	0.11	0.18	0.28
4	0.00	0.05	0.05	0.07	0.11	0.18	0.29
5	0.00	0.00	0.00	0.07	0.11	0.14	0.35
mean	**0.00**	**0.03**	**0.03**	**0.07**	**0.12**	**0.19**	**0.36**
SD	**0.00**	**0.03**	**0.03**	**0.01**	**0.01**	**0.04**	**0.08**
median	**0.00**	**0.05**	**0.05**	**0.07**	**0.11**	**0.14**	**0.35**
Rats	**OVX**						
	D 0	D 1	D 3	D 7	D 14	D 21	D 28
1	0.00	0.07	0.11	0.14	0.21	0.36	0.72
2	0.00	0.07	0.11	0.14	0.18	0.39	0.65
3	0.00	0.05	0.07	0.11	0.21	0.32	0.51
4	0.00	0.07	0.14	0.18	0.34	0.49	0.93
5	0.00	0.05	0.07	0.11	0.28	0.43	0.83
mean	**0.00**	**0.06**	**0.10**	**0.14**	**0.24**	**0.40**	**0.73**
SD	**0.00**	**0.01**	**0.03**	**0.03**	**0.07**	**0.07**	**0.16**
median	**0.00**	**0.07**	**0.11**	**0.14**	**0.21**	**0.39**	**0.72**
Rats	**OVX+ZOL**						
	D 0	D 1	D 3	D 7	D 14	D 21	D 28
1	0.00	0.00	0.00	0.05	0.11	0.14	0.22
2	0.00	0.00	0.00	0.05	0.11	0.15	0.25
3	0.00	0.05	0.05	0.07	0.11	0.18	0.36
4	0.00	0.05	0.05	0.07	0.14	0.18	0.36
5	0.00	0.05	0.05	0.07	0.14	0.18	0.42
mean	**0.00**	**0.03**	**0.03**	**0.06**	**0.12**	**0.17**	**0.32**
SD	**0.00**	**0.03**	**0.03**	**0.01**	**0.02**	**0.02**	**0.08**
median	**0.00**	**0.05**	**0.05**	**0.07**	**0.11**	**0.18**	**0.36**

Table 2

Comparisons of the area, depth and volume of OIRR between the control, OVX and OVX+ZOL groups in the mesial (M), disto-buccal (DB) and disto-palatal (DP) roots.

Rats	Area of OIRR (x10^2 µm^2)								
	M			DB			DP		
	CONT	OVX	OVX + ZOL	CONT	OVX	OVX + ZOL	CONT	OVX	OVX + ZOL
1	11.37	23.82	3.18	11.44	33.87	5.16	16.99	33.49	2.49
2	12.51	21.89	18.53	13.85	35.52	12.75	14.28	35.12	17.73
3	14.81	23.31	5.29	7.75	30.99	13.43	15.45	28.98	18.47
4	4.51	18.84	11.24	11.51	40.04	3.23	19.52	39.19	5.20
5	4.29	18.75	4.26	3.41	32.36	4.66	9.95	26.52	7.15
mean	**9.50**	**21.32**	**8.50**	**9.59**	**34.56**	**7.85**	**15.24**	**32.66**	**10.21**
SD	**4.82**	**2.41**	**6.42**	**4.09**	**3.50**	**4.85**	**3.55**	**5.02**	**7.40**
median	**11.37**	**21.89**	**11.24**	**11.44**	**33.87**	**11.27**	**15.45**	**33.49**	**15.28**
Rats	**Depth of OIRR (µm)**								
	M			**DB**			**DP**		
	CONT	**OVX**	**OVX + ZOL**	**CONT**	**OVX**	**OVX + ZOL**	**CONT**	**OVX**	**OVX + ZOL**
1	7.37	40.24	4.98	18.50	56.82	16.40	16.89	45.43	10.05
2	4.12	13.20	4.96	9.11	54.93	13.90	12.71	44.45	13.51
3	6.28	32.94	5.75	11.55	46.69	16.89	14.99	41.70	17.16
4	6.98	37.09	5.73	18.50	39.32	11.38	16.89	36.82	12.95
5	4.13	37.79	7.20	9.30	37.41	10.11	16.97	36.05	12.39
mean	**5.78**	**32.25**	**5.72**	**13.39**	**47.03**	**13.74**	**15.69**	**40.89**	**13.21**
SD	**1.56**	**10.97**	**0.91**	**4.76**	**8.81**	**2.99**	**1.86**	**4.30**	**2.57**
median	**6.28**	**37.09**	**5.73**	**11.55**	**46.69**	**13.90**	**16.89**	**41.70**	**12.95**
Rats	**Volume of OIRR (10^4 µm^3)**								
	M			**DB**			**DP**		
	CONT	**OVX**	**OVX + ZOL**	**CONT**	**OVX**	**OVX + ZOL**	**CONT**	**OVX**	**OVX + ZOL**
1	0.84	9.59	0.16	2.12	19.24	0.85	2.87	15.21	0.25
2	0.52	2.89	0.92	1.26	19.51	1.77	1.81	15.61	2.40
3	0.93	7.68	0.30	0.90	14.47	2.27	2.32	12.08	3.17
4	0.31	6.99	0.64	2.13	15.74	0.37	3.30	14.43	0.67
5	0.18	7.09	0.31	0.32	12.11	0.47	1.69	9.56	0.89
mean	**0.56**	**6.85**	**0.47**	**1.34**	**16.21**	**1.15**	**2.40**	**13.38**	**1.47**
SD	**0.33**	**2.45**	**0.31**	**0.79**	**3.17**	**0.84**	**0.69**	**2.54**	**1.25**
median	**0.52**	**7.09**	**0.31**	**1.26**	**15.74**	**0.85**	**2.32**	**12.08**	**0.89**

Table 3

The total volume of OIRR in three roots including the mesial (M), disto-buccal (DB) and disto-palatal (DP) roots comparing between the control, OVX and OVX+ZOL groups.

Rats	Total volume of OIRR in three Roots (x10^4 µm^3)		
	CONT	OVX	OVX+ZOL
1	5.82	44.04	1.25
2	3.59	38.01	5.09
3	4.14	34.23	5.74
4	5.74	37.16	1.69
5	2.18	28.75	1.66
mean	**4.30**	**36.44**	**3.09**
SD	**1.80**	**8.15**	**2.39**
median	**4.14**	**37.16**	**1.69**