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ORIGINAL ARTICLE

Effects of estrogen deficiency on tooth movement after force application: an experimental study in ovariectomized rats

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Abstract

Objective. The aim of this study was to investigate the effects of estrogen deficiency on tooth movement in ovariectomized rats. ***Material and methods.*** Forty-two adult female Sprague-Dawley rats were assigned at random to one of the following groups: test group ($n=20$), ovariectomized rats (or estrogen-deficient rats); control group ($n=22$), non-ovariectomized rats. Two months after ovariectomy, expansion springs exerting 10 g of force were inserted between the upper central incisors in both groups. The amount of movement was measured daily until tooth movement began and then at intervals of 3 days. The rats were sacrificed 18 days after applying the expansion spring and histomorphometric analysis was performed along the left upper central incisor root towards the apex of the alveolar bone. ***Results.*** The amount and speed of movement was observed to be greater in ovariectomized rats. On histomorphometric analysis, osteoblast and osteocyte counts on the pressure side were higher in the non-ovariectomized group than in the ovariectomized group ($p<0.001$). In contrast, the osteoclast count was significantly higher ($p<0.01$) in the ovariectomized group than in the non-ovariectomized group. The osteoblast and osteocyte counts were significantly higher ($p<0.001$) on tension side in the non-ovariectomized group than in the ovariectomized group. ***Conclusion.*** Estrogen deficiency increased orthodontic tooth movements but counts of osteoblasts, which are responsible for new bone formation, were lower in regions of tension and of pressure.

Introduction

Bone loss occurs throughout the early phase of estrogen deficiency, and is accompanied by increased bone turnover. This phenomenon has been demonstrated in a number of radiographic studies with rats, monkeys, and humans [1–5]. Wronsky et al. [6] observed trabecular bone loss in ovariectomized rats, and determined that this was accompanied by increased longitudinal bone growth and accelerated bone resorption and formation. Tanaka et al. [7] demonstrated that estrogen deficiency in ovariectomized rats caused osteoporotic changes in the alveolar bone, and alveolar bone thinning in the 1st molar interradicular septa.

Tooth movement is achieved through the resorption and formation of bone in response to compression and distraction [7]. Therefore, all metabolic and hormonal changes affecting bone turnover may affect tooth movements. One of the most important of these changes is postmenopausal osteoporosis, which may occur naturally or otherwise [8]. Although it is expected that reduced levels of estrogen and increased bone turnover in the postmenopausal period will affect the form [7,8] and results of orthodontic therapy, few studies of the effects of estrogen on tooth movements have been performed [9,10].

The aim of this study was to investigate the effects of estrogen deficiency on tooth movement in ovariectomized rats. We hypothesized that estrogen deficiency would increase the extent and speed of tooth movement because of accelerated bone turnover.

Material and methods

The study was approved by the Ethics Committee of the Dicle University Medical Faculty. Non-pregnant adult [90 ($\pm$10) days old] female Sprague-Dawley

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rats weighing 200–250 g (bred in the Animal Research Laboratory of the Dicle University Health Sciences Institute) were used in this study. Forty-two rats were assigned randomly to either the test group of 20 ovariectomized or estrogen-deficient rats; or the control group of 22 non-ovariectomized rats.

The rats were kept in standard rat cages in an air-conditioned (18–26°C) room where the humidity was controlled at 40–60% with the help of a hygrometer. The ovariectomized rats in the test group were housed in 5 cages with 4 rats in each. The non-ovariectomized rats of the control group were housed in 3 cages containing 4 rats and 2 cages containing 5 rats. Each rat was fed with 10–15 $g \cdot day^{-1}$ of a standard rat pellet diet.

Rat osteoporosis model and experimental tooth movement

General anesthesia was induced by intraperitoneal injection of 50 mg kg^{-1} ketamine hydrochloride (Ketalar, Eczacıbaşı, Lüleburgaz, Turkey) and 5 mg kg^{-1} xylazine hydrochloride (Rompun, Bayer, Istanbul, Turkey). Bilateral ovariectomy was performed and the excised tissues were histopathologically verified as ovarian. The success of ovariectomy was confirmed by observing marked atrophy of the uterine horns.

Ovariectomy was followed by a resting period of 2 months to allow the full effect of estrogen deficiency [7,11]. At the end of 2 months, anesthesia was induced by the same method, and an appliance exerting force to widen the space between the upper central incisors was fitted to both groups. The appliance used in this study was an expansion spring made of 0.012 inch stainless steel wire and modified as described by Storey [12] and by Stark and Sinclair [13]. The appliance was fitted for each rat with the aid of Tweed pliers and Mathieu pliers so that it would be 1 cm in size. After the appliance was activated to exert 10 g of force monitored with a dynamometer (Figure 1), it was inserted into a hole made in the gum at a distance of 1.5 mm from the crown of the incisor in the vestibulo-palatal direction (Figure 2).

Measurement of tooth movements

Electronic digital calipers (150 mm HS/R3/1A; Knuth Machine Tools, KG, Wasbek, Germany) with an accuracy of ± 0.01 mm were used to measure the amount of tooth movement. The distance between the upper incisors was measured directly with a digital compass at a distance of 2 mm from the gum. Measurements were made daily until tooth movement began, and then at intervals of 3 days. Tooth movement was measured twice a day at different times by the same researcher (S.G.A.) and mean values were recorded.

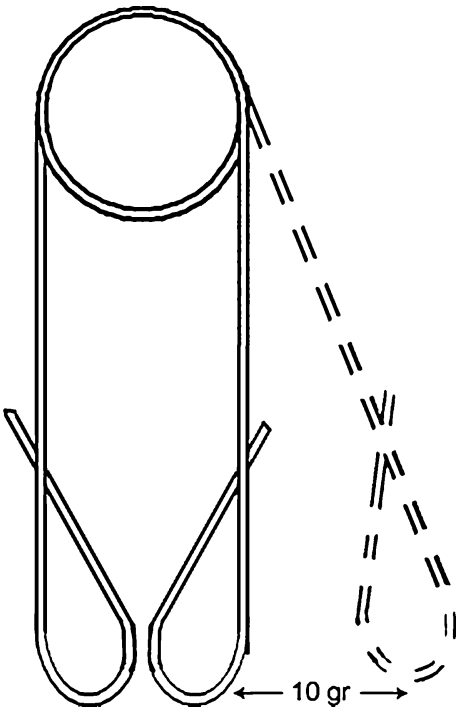

Figure 1. Activation of the spring.

Histomorphometric analysis

The histomorphometric parameters of tension (mesial) and pressure (distal) regions were measured. For standardization, the same region was excised in all rats, along the left upper central incisor root towards the apex of the alveolar bone on the mesial and distal sides (3 mm above the apex towards the crown). Excised tissues were fixed in 10% neutral formalin solution, then decalcified in 5% formic acid. After routine histological follow-up, the specimens were embedded in paraffin blocks. The alveolar bone was cut with a Leica rotation microtome (RM 2125, Nussloch, Germany) and 5 serial sections were made for each animal with 100 μm distance and 5 μm thickness [14]. Histological sections were stained with hematoxylin and eosin, and with hematoxylin and Van Giesson.

Tissue micrographs were taken with a binocular research microscope, a digital camera (Nikon Eclipse-400 and Nikon Coolpix 5000; Chiyoda-ku, Tokyo, Japan), and a personal computer. The application used was the New Vision area measurement programme (1 mm^2) in the Windows 98 operating system. Counts of osteoblasts, osteocytes, and osteoclasts in 1 mm^2 in the alveolar bone were done in the same region in each animal.

Statistical analysis

The ovariectomized group and the non-ovariectomized group were compared with regard to both amount of movement (final movement amount and comparison between the measurements of two groups in observation days) and histomorphometric

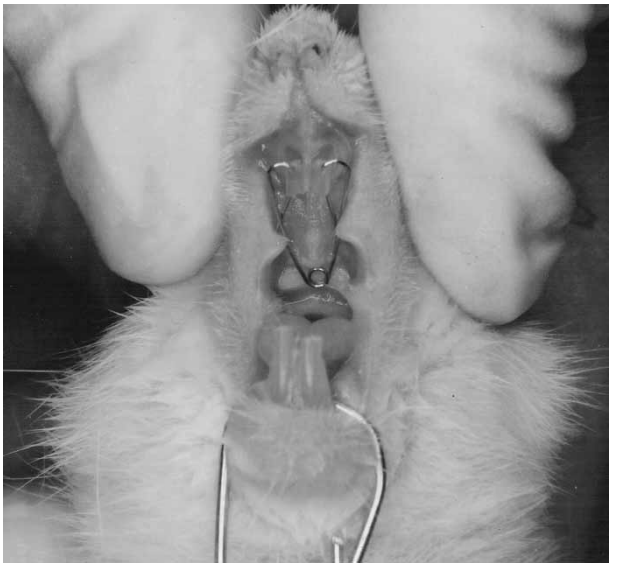

Figure 2. Application of the spring.

difference using the Mann-Whitney U-test. The measurements were repeated by the same researcher (S.G.A.) at different times in order to determine the individual measurement error in measuring the final amount of tooth movement in both groups. The intra-observer measurement error was 0.05 mm in the ovariectomized test group and 0.04 mm in the non-ovariectomized control group. The reliability coefficient was 0.99 for both groups.

Results

Measurement of tooth movement

In both groups, tooth movement was observed to begin on the third day. Throughout the following days, however, it was observed that the speed and amount of tooth movement was greater in the ovariectomized group (Figure 3). The difference between the groups was significant by day 6 ($p < 0.05$) and was even greater ($p < 0.001$) for days 12, 15, and 18.

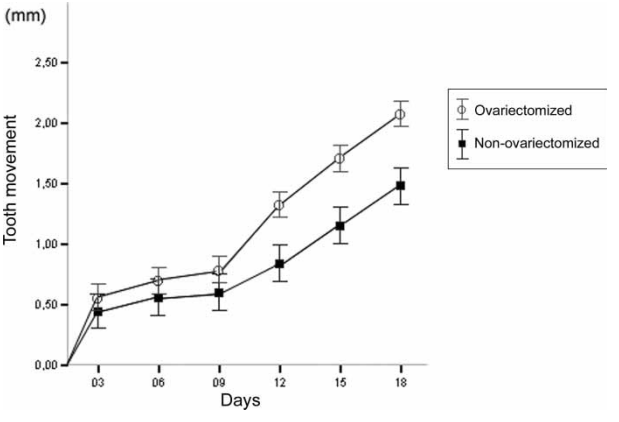

Figure 3. Experimental tooth movement in mm (mean ± standard deviation) during the experimental period in the ovariectomized test group and in the non-ovariectomized control group.

The comparison of tooth movement on the final day of observation was done using the Mann-Whitney U-test. The amount of movement was significantly ($p < 0.001$) higher in the ovariectomized group (mean (SD) 2.07(0.24) mm) than in the non-ovariectomized group (1.48 (0.38) mm).

Histomorphometric analysis

Cell counts and the comparison of the difference between these counts in ovariectomized and non-ovariectomized groups in the tension and pressure regions (Mann- Whitney U test results) are shown in Table I.

Discussion

Tooth movements are achieved through the resorption of bone and formation of bone in response to compression and distraction forces. For this reason, factors affecting bone structure and destruction can be expected to affect tooth movement and, consequently, therapeutic outcomes. In the literature, there are different views on the effects of estrogen deficiency on the formation of new bone and the strength of the newly formed bone tissue [1,6,7,15]. There have been many biomechanical and histological studies reported showing that estrogen deficiency affects the strength of newly formed bone [1,6,7,15–18] and, to our knowledge, there is only one report showing the opposite [11]. There are two points of view regarding the mechanism underlying the effect of estrogen deficiency on new bone formation and structure. The first concerns the negative osteo-inductive effects of estrogen deficiency. Cesnjaj et al. [1] determined that osteogenesis decreased in ovariectomized rats and that chondrogenesis was delayed. Those authors hypothesized that estrogen deficiency alters the production of osteo-inductive proteins such as osteogenin and bone morphogenetic protein, and in this way disrupts bone matrix formation. This mechanism explains the effect of estrogen deficiency on the early stage of new bone formation. The second point of view is that estrogen deficiency increases bone turnover, causing osteoporosis in both newly formed bone and old bone [5]. Kubo et al. [11] suggested that this mechanism may have a negative effect on the late stage of bone re-establishment. In contrast, in a biomechanical study, Walsh et al. [19] determined that, although new bone was less durable in ovariectomized rats, this difference decreased in the late stage. Tanaka et al. [7], in a study with ovariectomized rat models, found that estrogen deficiency affected trabecular bone volume and the number of trabeculae in the alveolar bone significantly. In the present study, it was observed that tooth movements in the osteoporotic model group were greater and more rapid than in the

Table I. Cell counts and the comparison of the difference between these counts in ovariectomized and non-ovariectomized groups in the tension and pressure regions. (Mann- Whitney U test results)

REGIONS	Cells	Groups	Groups n	Minimum	Maximum	Median	Mean	SD	Mean Difference over-nonover.	P
DISTAL (PRESSURE)	Osteoblast	Ovariectomized	20	4	8	5	5.7	1.3	−2.06	***
		Non-ovariectomized	22	6	10	8	7.8	1.3		
	Osteocyte	Ovariectomized	20	5	8	6	6.5	1.1	−3.53	***
		Non-ovariectomized	22	7	13	10	10.1	2.1		
	Osteoclast	Ovariectomized	20	2	5	4	3.5	0.9	1.06	**
		Non-ovariectomized	22	1	4	2	2.5	0.9		
MESIAL (TENSION)	Osteoblast	Ovariectomized	20	2	5	3	3.6	1.1	−4.46	***
		Non-ovariectomized	22	5	11	8	8.1	2.2		
	Osteocyte	Ovariectomized	20	3	6	4	4.3	1	−3.46	***
		Non-ovariectomized	22	6	9	8	7.7	1		
	Osteoclast	Ovariectomized	20	1	4	2	2.4	0.9	0.53	NS
		Non-ovariectomized	22	1	3	2	1.9	0.7		

Abbreviations: SD, standard deviation; P, probability; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, $P > 0.05$ (indicates not significant).

non-ovariectomized group, which can be explained by estrogen deficiency increasing bone turnover, since increased bone turnover enhances the response of bone to compression and distraction forces, and this in turn accelerates tooth movements. The clinical significance of this finding is that estrogen deficiency might accelerate the orthodontic movements.

According to the histomorphometric data of this study, the count of osteoblasts, which are responsible for new bone formation, was lower in the ovariectomized test group than in the non-ovariectomized control group. This finding supports the report by Kubo et al. [11] that estrogen deficiency has a negative effect on the late stage of bone formation. In the first stage of orthodontic therapy, the aim is to correct any orthodontic abnormality by tooth movements achieved by various mechanical devices (active orthodontic stage). The second, and the more important, stage of orthodontic therapy aims to keep the correction stable and to prevent relapse (stabilization stage). It is essential to have a bone with normal density in the tension side to prevent relapse.

Our findings show that, although estrogen deficiency may help achieve a rapid correction, it may negatively affect the maintenance of this correction. In our opinion, these findings have two consequences for clinical application: the first is that consolidation therapy will probably take longer in estrogen-deficient patients, and the second is that relapse and therapeutic failure will be more common in these patients. A survey of the literature found no clinical data to support or disprove this opinion. Miyajima et al. [9], in the report of a case treated in the postmenopausal period, stated that the orthodontic tooth movements were slow, but that the patient had been taking estrogen hormone therapy regularly for 3 years, and they suggested that this was the reason for the slow tooth movement. In a study with ovariectomized rats, Adachi et al. [20] found that bisphosphonates (risedronate) prevented relapse, concluding that the clinical use of bisphosphates may be useful for anchorage and the maintenance of correction. Nonetheless, our findings and assumptions need to be supported by more clinical studies.

Conclusions

In this study carried out with an ovariectomized rat model, we observed that estrogen deficiency increased orthodontic tooth movement, and that the count of osteoblasts, which are responsible for the formation of new bone and regeneration of bone, was lower in the ovariectomized group than in the non-ovariectomized group in both tension and pressure sides. These findings indicate that estrogen deficiency in the postmenopausal period may facilitate the correction phase of orthodontic therapy, but

may complicate and increase the duration of the maintenance phase, as well as increase the relapse rate.

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