Original Article

Effect of Ovariectomy on Orthodontic Tooth Movement in Rats

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Abstract

Background and Aim: Estrogen affects the metabolism in females. This study aimed to assess the effect of absence of estrogen on orthodontic tooth movement in rats.

Materials and Methods: In this animal study, 55 rats were randomly divided into four groups. Group 1 (n=10) had intact ovaries. In group 2 (n=15), ovaries were intact but the rats were subjected to stress due to tooth preparation. In group 3 (n=15), the ovaries were intact but the rats were subjected to stress due to tooth preparation and surgical manipulation. In group 4 (n=15), ovaries were removed and the rats were subjected to stress due to tooth preparation and surgical manipulation. Orthodontic appliances were placed after 14 days in groups 2 to 4. All groups except for group 1 were subjected to mesial movement of maxillary right first molar using NiTi closed coil spring applying 60g load for 21 days. The rats were sacrificed at 21 days and the amount of orthodontic tooth movement was measured using a feeler gauge. Data were analyzed using ANOVA.

Results: The mean orthodontic tooth movement was 1.18±0.2 mm in group 4, which was significantly greater than that in group 3 (0.47±0.18 mm) and group 2 (0.22±0.07 mm) ($P<0.001$).

Conclusion: Absence of estrogen can significantly accelerate orthodontic tooth movement in rats.

Key Words: Tooth Movement Techniques, Gonadal Steroid Hormones, Rats

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Introduction

Bone remodeling is the basis of orthodontic treatment. When pressure is applied to teeth for a long period of time, bone is selectively resorbed in some areas and deposited in some other areas. This results in movement of tooth along with its attached structures in bone. Since the bone response is mediated by the periodontal ligament, tooth movement is primarily based on the periodontal ligament. According to the classic theory of tooth movement or the tension-pressure theory, change in blood flow of the periodontal ligament immediately changes the chemical environment causing cell differentiation and activity. Tooth movement is then initiated by the activity of osteoclasts and osteoblasts [1].

Nowadays, orthodontic treatment is no longer limited to the growth age, and about 20 to 30% of orthodontic patients are adults especially women [2]. Therefore, orthodontists must be aware of factors affecting tooth movement as well as systemic, metabolic and hormonal confounders, which are often overlooked in adult patients [3]. It has been reported that sex hormones namely

estrogen, progesterone and testosterone play a fundamental role in bone metabolism and are necessarily required for development, maturity and preservation of bones [4]. Since the teeth are located in bony sockets, the bone undergoes remodeling during orthodontic movement. These hormones affect bone remodeling and may modify tooth movement [4]. A previous study showed that increase in duration of treatment increases the risk of enamel demineralization, root resorption, dental caries and periodontal disease. Therefore, the effect of these hormones on dentition should be taken into account since these hormones decrease the speed of bone remodeling and prolong the course of treatment [5].

On the other hand, serum level of estrogen and progesterone decreases in menopaused women and may result in osteoporosis [4]. Tanaka et al. discussed that absence of estrogen in ovariectomized female rats decreased the bone mass and resulted in thinning of bone in the inter-radicular septum of first molar teeth [6]. Since bone remodeling occurs during orthodontic tooth movement, role of sexual hormones in bone regeneration and remodeling has been extensively studied [4]. A previous study showed that bone resorption occurred 14 to 30 days after resection of the ovaries [7]. However, studies on the effects of hormones on tooth movement are limited [4]. Poosti et al. demonstrated that progesterone had no significant effect on tooth movement in short-term but its long-term injection significantly decreased tooth movement [8]. Another study showed that changes in serum estrogen can change serum markers of bone regeneration [4]. Therefore, orthodontic treatment must be adjusted based on the menstrual cycle in females [2]. Haruyama et al. showed that orthodontic tooth movement significantly depended on the menstrual cycle in females such that orthodontic tooth movement was maximal after ovulation and was minimum before ovulation. They added that orthodontic tooth movement had an inverse correlation with serum level of estrogen in rats [9]. Another study on ovariectomized rats indicated that 18 days after removal of ovaries, absence of estrogen facilitated tooth movement but number of osteoblasts at the tension and pressure sites unexpectedly decreased [7]. Considering the existing controversies and absence of a comprehensive study on the effect of female sex hormones on orthodontic tooth movement, this study aimed to assess the effect of absence of female sex hormones on orthodontic tooth movement. Histomorphometric effect of absence of female sex hormones on the alveolar bone metabolism during orthodontic tooth movement was also evaluated.

Materials and Methods

In This animal study was conducted in Shiraz University of Medical Sciences and approved in the ethics committee of this university (code: 91-4433). This study was conducted on 55 six-week old Sprague-Dawley rats with a mean weight of 160±20 g, which were maintained in standard conditions in terms of humidity, ventilation, temperature and light with ad libitum access to food and water. The rats were randomly divided into four groups using a table of random numbers. Group 1 included 10 rats and the remaining three groups included 15 rats each; four rats were expired during the study and the remaining 51 were subjected to tooth preparation stress, surgical manipulation stress, and ovariectomy. Rats were fed soft food daily. Ketamine hydrochloride (90 mg/kg; Ketaject; Clipper Distributing Company LLC, St. Joseph, MO, USA) and xylazine (8 mg/kg; Rumpon; Bayer Animal Health, Kansas, USA) were injected intramuscularly in the biceps femoris muscle for anesthesia. Group 1 included 10 female rats with intact ovaries and they received no stress. They did not receive orthodontic appliances either. Orthodontic appliances were placed in all other groups and the incisal edge of the mandibular incisors was cut by 2-3 mm. Group 2 was subjected to tooth preparation stress and group 3 was subjected to tooth preparation and surgical manipulation stress; however, their ovaries were not resected and were only manipulated at the connection of the Fallopian tubes and ovaries with a hemostat. In group 4, the rats were subjected to tooth preparation and surgical manipulation stress and they were ovariectomized bilaterally. According to previous studies [6,10,11], 14 days after surgery, orthodontic appliances were placed on teeth. Load application and assessment of tooth

movement in this study were similar to those in the study by Bridges et al [12]. Medium-size NiTi closed coil spring measuring 0.03x0.01" (Nitinol, 3M Unitek, Monrovia, CA, USA) and 9 mm in length was connected from both sides to the maxillary right first molar and maxillary right incisor by 0.10" ligature wire and fixed with composite resin. The coil spring was activated by 1 mm in 30 days to apply 60 g load to teeth (American Orthodontics, Sheboygan, Wisconsin, USA). To prevent aspiration and suffocation of rats when rinsing their mouth, saliva ejector was used. Due to weight loss because of placement of appliance, the rats were weighed twice a week. In all groups, the rats were sacrificed 21 days later with ether. The distance between the maxillary right first and second molars was measured before removal of coil spring using a feeler gage with 0.01 mm accuracy. To prevent the wedging effect when placing the feeler gage between the two teeth, the distance between the distal surface of the third molar tooth in the right side to the mesial surface of the first molar in the right side of the maxilla was measured by a digital caliper with 0.01 mm accuracy (Mitutoyo, Tokyo, Japan) before and after placement of feeler gage and care was taken to equalize both distances. To eliminate the effect of difference in measurement of distance by more than one operator, all measurements were made by the same operator [13]. Measurements were repeated on 20 randomly selected rats to test intra-observer reliability, which was found to be 96% and confirmed the reliability of measurements.

Data were analyzed using SPSS version 15. One-sample Kolmogorov-Smirnov test was used to test the normality of data and assess the difference in the mean movement of teeth in group 1 (no appliance) and other groups. One-way ANOVA was applied to compare the distance among the three groups subjected to orthodontic loads (groups 2-4). Tukey's post hoc test was applied for pairwise comparisons made in three groups under orthodontic load. It should be noted that since in group 1 (no appliance group) the mean and standard deviation of tooth movement was zero, logarithmic conversion of this value and its inclusion in ANOVA and Tukey's test could confound the results.

Results

Assessment of normal distribution of data in each group by use of one-sample Kolmogorov-Smirnov test showed that data were normally distributed in all groups ($P>0.05$). Since difference in standard deviation of groups could confound ANOVA, to compress the data and equalize the standard deviation in the three groups, their log (Ln) was used. Table 1 shows the mean and standard deviation of orthodontic tooth movement in the four groups. Assessment of orthodontic tooth movement in the groups showed a significant difference in the mean amount of tooth movement among the groups ($P<0.001$). The mean log (Ln) of orthodontic tooth movement was not the same in all three groups. Therefore, the total mean of movement was different among the three groups. The least orthodontic tooth movement was noted in group 2 with a mean of 0.22 mm and the highest orthodontic tooth movement was noted in group 4 (ovariectomized group) with a mean of 1.18 mm.

Diagram 1 compares the mean log of tooth movement in the three groups. The mean log of orthodontic tooth movement was below zero in groups 2 and 3 because the mean amount of tooth movement in these groups was between 0 and 1 and the log of the values between 0 and 1 is a negative value. Diagram 1 shows that the more negative the value, the less the tooth movement would be. The positive value of the mean log of tooth movement in ovariectomized group (+0.1516) showed greater amount of tooth movement in this group.

The results of Tukey's post hoc test for pairwise comparison of the groups showed that:

1.Orthodontic tooth movement in group 2 (0.22 mm) was significantly less than the mean value in other groups ($P<0.001$).

The mean amount of orthodontic tooth movement in ovariectomized group (1.18 mm) was significantly higher than the mean orthodontic tooth movement in the other two groups ($P<0.001$).

3.The mean amount of tooth movement in surgical manipulation group (0.47 mm) was significantly higher than the mean value in group 2 ($P<0.001$).

Discussion

The results of this study showed that absence of estrogen and progesterone significantly increased

Table 1. Comparison of the mean orthodontic tooth movement in the groups using ANOVA

Group	--	Number	Mean (mm)	Standard deviation
1	No appliance	10	0	0
2	Tooth preparation stress	14	0.221	± 0.075
3	Tooth preparation and surgical manipulation	14	0.475	± 0.181
4	Ovariectomized	13	1.180	± 0.207

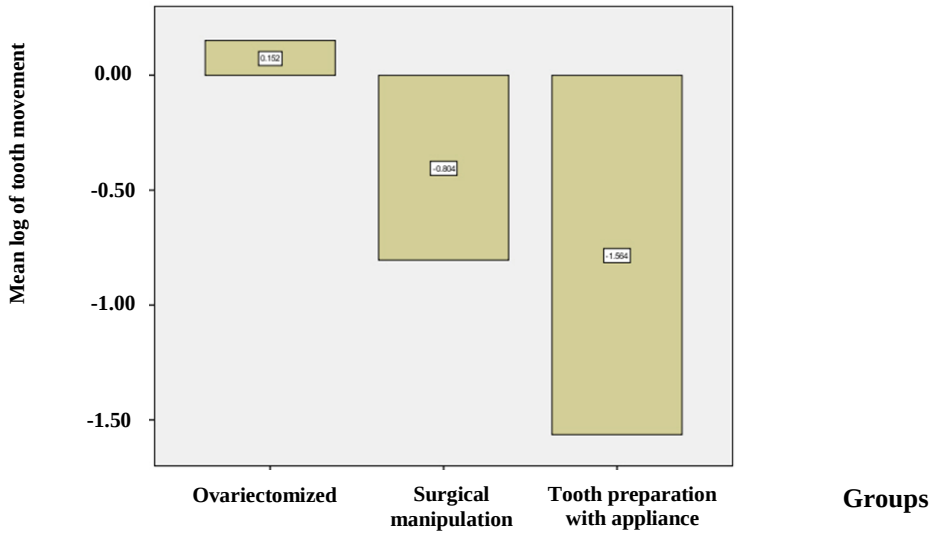

Diagram 1. Mean log of tooth movement in different groups. More negative values indicate less

the orthodontic tooth movement in rats. Use of a wide range of forces is the most important factor causing differences in the results of studies inducing orthodontic tooth movement. However, no consensus has been reached on the optimal orthodontic load for this purpose [11,14,15]. Based on previous studies, 60 g load was used in our study for mesial movement of the maxillary first molar in rats [16]. On the other hand, duration of studies on properties and the biological response of tooth movement must be at least two weeks [15]. In our study, 21 days were allowed to assess the effect of ovariectomy and subsequent absence of sexual hormones on tooth movement because this time period is suitable for assessment of bone remodeling process and orthodontic tooth movement and causes minimum injury due to load application. Therefore, this time point is suitable for orthodontic visit and reactivation of appliances. It has been shown that orthodontic appliances should not be reactivated sooner than three weeks, and seven to 14 days of time is required for the undermining resorption to occur. On the other hand, if the device is spring-type, similar to NiTi coil spring used in this study, light loads would result in direct resorption and there would be no need for reactivation of device.

As mentioned earlier, studies on the effect of sexual hormones on tooth movement are limited. Yamashiro et al. evaluated46 rats in two groups. In the test group, both ovaries were completely resected similar to the current study but in the control group, ovaries were removed and replanted again in rats. No significant difference was noted in orthodontic tooth movement between the two groups up to day 12. But from day 12 to 21, orthodontic tooth movement was greater in ovariectomized group. They used 0.016 inch wires applying 10 g load buccally to the first molars and activated them in specific time points. No tooth

movement in the first days may be due to the application of very light loads. In our study, daily monitoring was performed to ensure the accuracy of load application, and mesial movement of molar tooth was visible since the first days. Also, the difference in orthodontic tooth movement between ovariectomized group and other groups was greater in our study, which may be due to different devices used in the two studies. However, with 60 g controlled load applied with NiTi coil spring in our study, our results seem to be more reliable, which indicates significant effect of female sex hormones on bone metabolism in females.

Arslan et al. [10] evaluated 42 rats in two groups of ovariectomized and control. They aimed to assess the effect of osteoporosis on tooth movement. They showed that two months after ovariectomy, the rats developed osteoporosis. But in our study, the effect of sex hormones was evaluated prior to the osteoporosis phase and the results showed that these effects were significant. Arslan et al. [10] used a 0.012-inch expansion spring activated by pliers to apply 10 g load. This method of activation, despite load control with a gage, yields highly variable results. Nonetheless, orthodontic tooth movement in this group was higher than that in other groups since the rats were completely osteoporotic. Their findings were similar to ours in this respect. However, the spring used in their study applied intermittent load to teeth; it means that when the tooth moves, surface area of the load decreases but returns to the primary state after reactivation [1]. Their study was different from ours since we used a NiTi coil spring. Moreover, they made hard silicon impressions of teeth in rats for measurement of tooth movement. This can change the measurement results and also applies stress to rats. Our study also showed that stress created in rats accelerated orthodontic tooth movement. None of the studies on the effects of drugs and hormones on orthodontic tooth movement following load application (similar to the current study) have had a separate stress group; thus, further studies are required on this topic.

To accurately assess the effects of reduction or absence of sex hormones on tooth movement, it is suggested to use molecular blockers of these hormones or their specific antagonists to eliminate possible unknown effects of ovariectomy on tooth movement. Also, assessment of serum level of hormones with special kits is recommended. Considering the results of this study regarding the effect of sexual hormones on orthodontic tooth movement, it is suggested to assess the effect of oral contraceptives on orthodontic tooth movement because these tablets are extensively used by young women and may potentially slow down orthodontic tooth movement and prolong the course of orthodontic treatment.

The results of this study showed that in absence of estrogen and progesterone, orthodontic tooth movement was significantly greater compared to that in other groups. Also, we had a group, in which the connection area of the Fallopian tube and ovaries was surgically manipulated and the results in this group showed that surgically induced stress can affect orthodontic tooth movement such that orthodontic tooth movement in this group was significantly greater than that in group 2 (no surgical manipulation). These significant changes indicate that stress induced in rats can accelerate orthodontic tooth movement.

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