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Original Article

The effect of osteopenia on tooth movement in ovariectomized rats. An experimental study

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Abstract

Objective: The purpose of this experimental study was to investigate in depth the effects of osteopenia related to the rate, as well as to the quality of orthodontic tooth movement, by combining experimental ovariectomy and molar movement in rats. **Methods:** Twenty-four six-month-old female Wistar rats were used in this study. The animals were divided into two groups consisting of twelve animals each: Group A (control group) was subjected to orthodontic movement of the upper right first molars. Group B was subjected to orthodontic movement of the upper right first molar following bilateral ovariectomy. Ovariectomy was performed on the first experimental day and the upper right first molars were subjected to orthodontic forces 60 days post-ovariectomy, lasting for 14 days. **Results and Conclusion:** Direct inspections of the upper jaws, measurements of orthodontic movement of the upper right first molars of Group A and B, as well as histologic examinations of the alveolar bone in the upper right and left first molar regions, showed that osteopenia affects the rate of orthodontic tooth movement, as well as the quality of alveolar bone remodeling, in ovariectomized rats. Specifically, in the ovariectomized animals the alveolar bone of the non-loaded side showed extensive internal resorption, with large marrow cavities, whereas the alveolar bone of the loaded side was dense with almost no marrow cavity and frontal resorption on the surface. It appears that alveolar remodeling after the exertion of orthodontic forces follows the general paradigm of osteoporotic bone remodeling after loading.

Keywords: Bone, Osteopenia, Osteoporosis, Rat, Tooth Movement

Introduction

Postmenopausal osteoporosis represents the most common metabolic bone disease in women today. It is related to estrogen withdrawal and represents a severe public health problem[1]. It is also generally accepted that among the skeletal sites affected by bone loss, the maxilla and the mandible are also included, both in peri- and post-menopausal women[2,3] as well as in rodent models[4,5]. Changes in bone metabolism are known to influence orthodontic tooth movement in experimental studies[6,7].

Following orthodontic tooth movement, tissue reactions take place in the paradental tissues resulting in differentiated bone turnover. It is well accepted that after the loading of a tooth, alveolar bone formation occurs on the tension side of the movement, whereas resorption is observed on the pressure side[8-10]. The extent and quality of tooth movement are dependent upon the nature of the applied force, the biologic status of the involved tissues and the general health condition of the treated individual[9]. Mechanical and metabolic control of alveolar bone homeostasis may influence the extent, as well as the rate and the quality of orthodontic treatment. Various regulatory mechanisms have been described and several genes are involved in local alveolar bone modeling processes[10].

The purpose of this experimental research study was to investigate in depth the effects of osteopenia related to the

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Table I. Body weight (g) during the experimental period.

Group	Before ovariectomy (6 months of age)	Before force application (8 months of age)	Final (8 months + 14 days of age)
A (n=12)	254.53±19.47	269.32±21.32	271.46±21.73
B (n=12)	256.49±18.94	271.23±18.29	273.02±18.97

rate, as well as to the quality of orthodontic tooth movement, by combining ovariectomy and molar movement in adult female rats.

Materials and methods

a. Laboratory animals

The experiments took place in the Laboratory of Experimental Surgery and Surgical Research of the Medical School of the University of Athens, as well as in the Laboratories of Oral Pathology and Orthodontics of the Dental School of the University of Athens. Twenty-four six-month-old female Wistar rats were used in this study. The experimental animals were obtained from the Hellenic Pasteur Institute. All animals were housed according to national regulations in conformance to the EU Directive 86/609 in force at that time (permit no. K/3816/5-7-05), in a room with regulated light (12 hs dark/12 hs light), temperature (20±2°C), relative humidity (55±5%) and were fed with normal pellet diet. The animals were divided into two groups consisting of twelve animals each, as follows: Group A (control group) included 12 rats that were subjected to orthodontic movement of the upper right first molars. Group B included 12 rats that were subjected to orthodontic movement of the upper right first molars following bilateral ovariectomy. The initial mean weight of the animals in both groups was 255 g (Table I). All animals were weighed every third day thereafter.

b. Ovariectomy and orthodontic device

On the first experimental day, bilateral ovariectomies were performed following general anesthesia in the female rats of group B from a ventral approach, according to the procedure described by Waynforth[11]. Anesthesia was administered using xylazine hydrochloride (Rompun, Bayer AG, Leverkusen, Germany) at a dose of 5 mg/kg body weight and ketamine hydrochloride (Ketaset, Fort Dodge, Iowa, USA) at 90 mg/kg body weight intramuscularly.

Orthodontic rat molar movement was achieved by the application of a closed nickel-titanium coil spring (0.010 x 0.045 inches) extending from the upper right first molar to the upper right central incisor (Figure 1). The spring was applied to all rats 60 days after ovariectomy of group B. The coil spring was 1 cm in length and its activation for 0.25 cm produced a force of 60 gr*. Super elasticity of the nickel-titanium coil spring resulted in the exertion

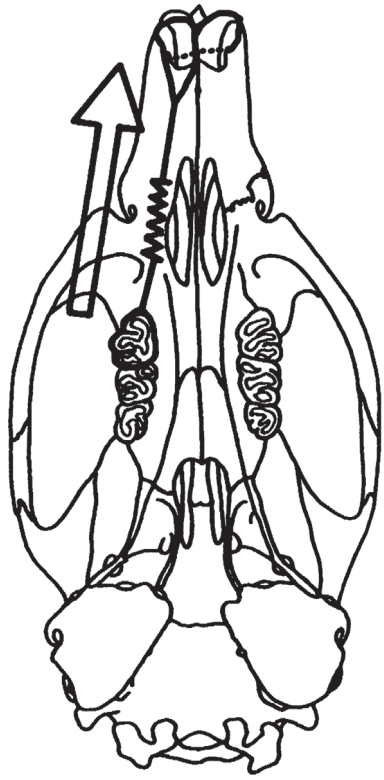

Figure 1. Schematic drawing of the experimental orthodontic appliance extending from the upper right first molar to the upper right central incisor.

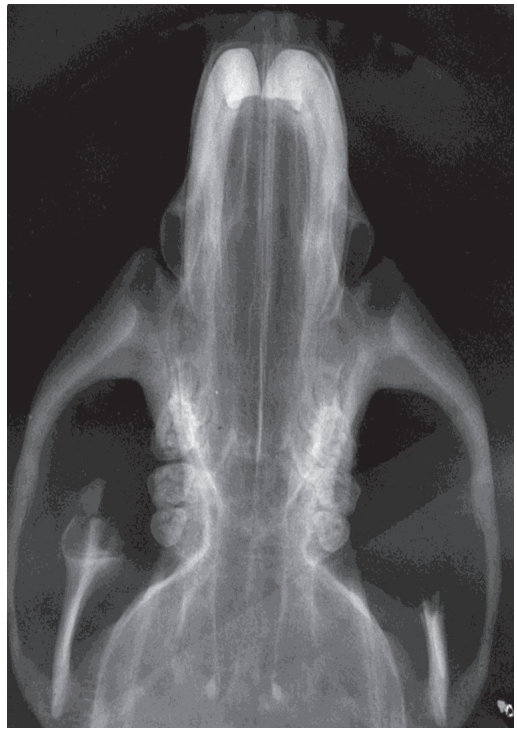

Figure 2. Special dorsoventral radiographs of the rat skull.

of a stable 60 gr* force on the molar teeth. The force magnitude of the appliance was measured with a precision force gauge (Correx Orthoorganizers, San Marcos, California) and the activation length of the appliance was measured with digital calipers (Orthoorganizers, San Marcos, California). The orthodontic force lasted for 14 days. The total experimental period was 74 days.

c. Tooth movement measurements

Molar movements were firstly observed in dental casts and measured in the special dorsoventral radiographs. In the present study, a split mouth design was chosen in order to eliminate possible compensantory remodeling effects on the molar alveolar region and also to limit the interanimal variation due to osteoporosis.

Dental casts were taken at the end of the experimental period for each animal. In the upper dental arch models, the distance between the most mesial point on the occlusal surface of the upper first molar and the most distal surface of the upper third molar was measured bilaterally with electronic calipers. Tooth movement was considered as the subtraction of the measured value of the treated side from that of the control side[7].

Dorsoventral radiographs were taken on day one and day 14 following the application of the orthodontic force. At the end of the experimental period, the mandible was dissected from the skull and thus special dorsoventral radiographs of the rest of the skull were taken of each animal (Figure 2). The radiographic method and procedure was similar to that described by Tsolakis et al.[12] The special dorsoventral radiographs were scanned and enlarged x9 to reduce measurement errors. The following landmarks were identified on each special dorsoventral radiograph to be used for the measurements of the molar movements (Figure 3):

Point A: the point where the line E meets the mesial occlusal surface of the upper right first molar.
Point B: the most mesial point on the occlusal surface of the upper right second molar.
Point C: the most distal point on the occlusal surface of the upper right third molar.
Line E: a line between points B and C extended on the occlusal surface of the upper right first molar.
Point A´: the point where the line E´ meets the mesial occlusal surface of the upper left first molar.
Point B´: the most mesial point on the occlusal surface of the upper left second molar.
Point C´: the most distal point on the occlusal surface of the upper left third molar.
Line E´: a line between points B´ and C´ extended on the occlusal surface of the upper left first molar.

In the special dorsoventral radiographs distances A-C and A´-C´ were measured with the built-in measurement tools of the viewbox 2 version 2.60 software[13]. Tooth movement was considered as the subtraction of the measured value A´-C´ from the measured value A-C.

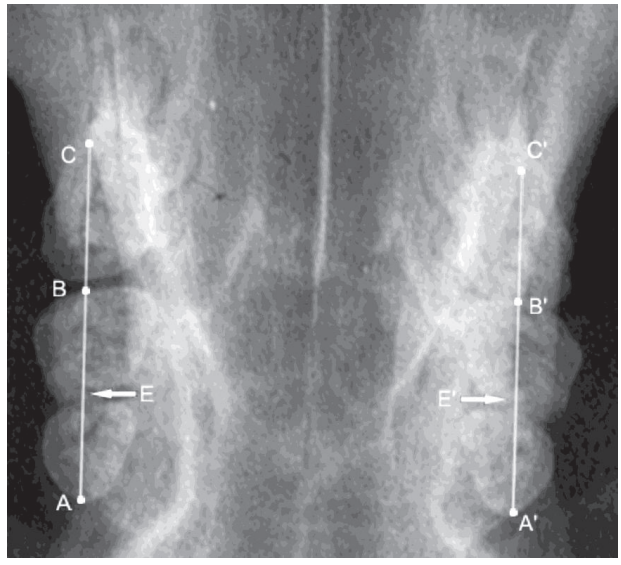

Figure 3. Landmarks and E line on the special dorsoventral radiograph.

d. Histological procedure and examination

Histological examinations of the alveolar bone and the peridontium surrounding the upper right and left first molars were performed. The interradicular areas of the same teeth were also examined histologically.

The specimens were cleaned from the surrounding soft tissues, fixed in 10% buffered formalin for 18 hours and decalcified in EDTA buffer for 6-8 weeks. Slices of 5 mm thickness were cut parallel to the first molar axis, including the first molar with the surrounding alveolar bone. The specimens were then dehydrated with ethanol and embedded in paraffin. From the 5 mm slices, histological sections of 4 to 5 μm thickness were obtained, stained with Hematoxylin and Eosin and were observed under transmitted light microscopy.

e. Statistical analysis

In tooth movement measurements of both experimental groups, the Wilcoxon test for unpaired measurements was applied. In addition, the error of the measurement method was estimated by replicating and double recording all special dorsoventral radiographs according to the formula $Se=\sqrt{(\Sigma d/2n)}$ proposed by Dahlberg (1940)[14].

Results

A. Tooth movement measurements

Direct inspections of the upper jaws, as well as of the dental arch models revealed a greater orthodontic movement of the upper right first molar for the animals of Group B (ovariectomized animals) (Figure 4), as compared to the orthodontic movement of the upper right first molar for the

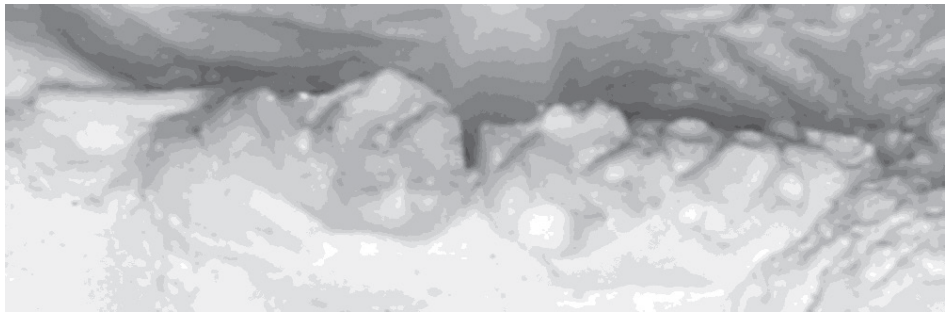

Figure 4. Dental arch model of a Group B (ovariectomized) animal.

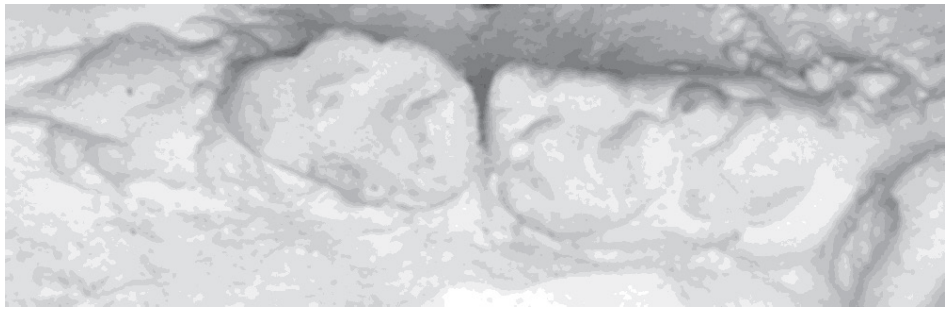

Figure 5. Dental arch model of a Group A (control) animal.

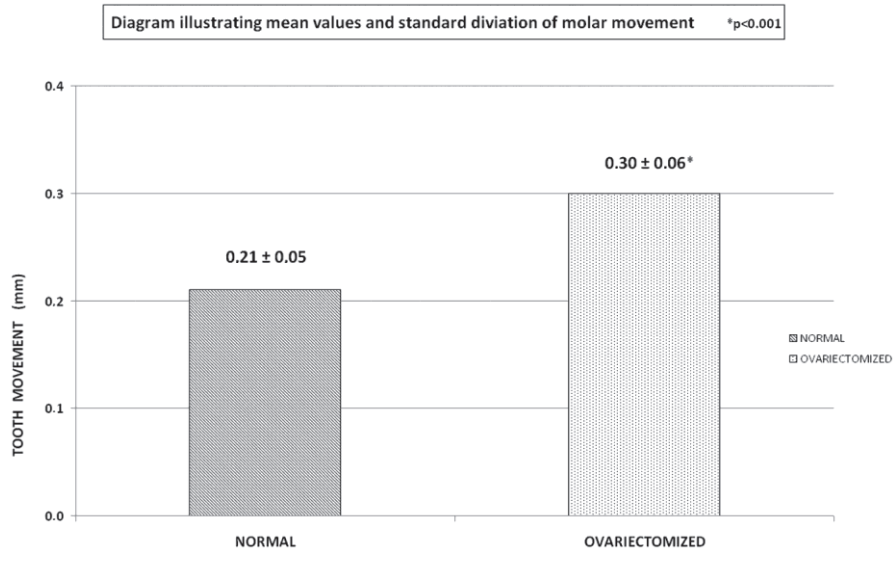

Figure 6. Histogram with mean values and standard deviations of molar movement in normal and ovariectomized groups.

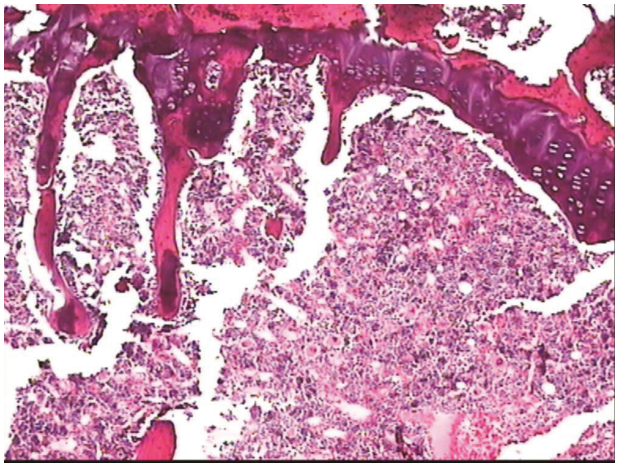

Figure 7. Trabecular bone of the tibia in an ovariectomized rat.

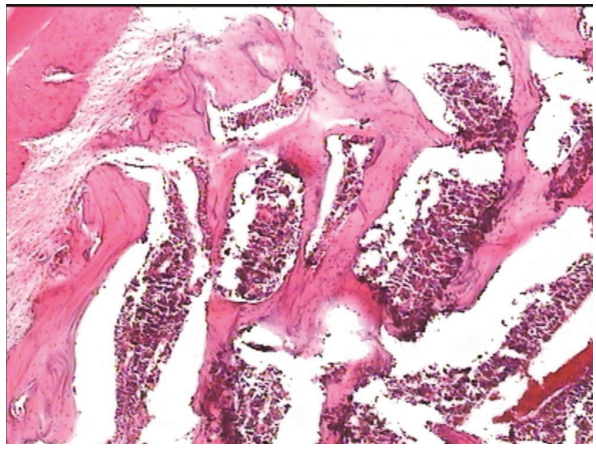

Figure 8. Alveolar bone in the apical region of a non-loaded first molar in an ovariectomized animal.

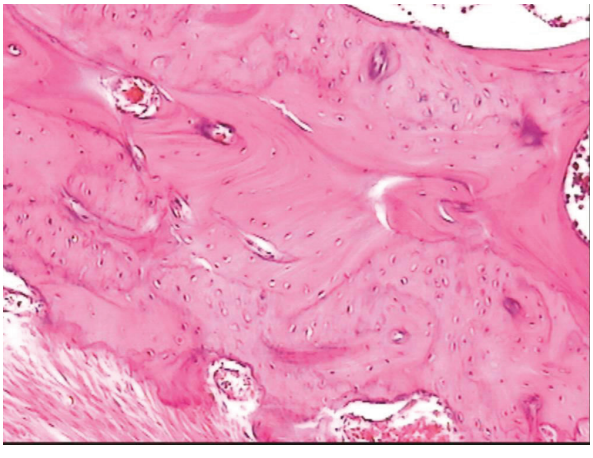

Figure 9. Alveolar bone in the apical area of a non-loaded first molar in a control animal.

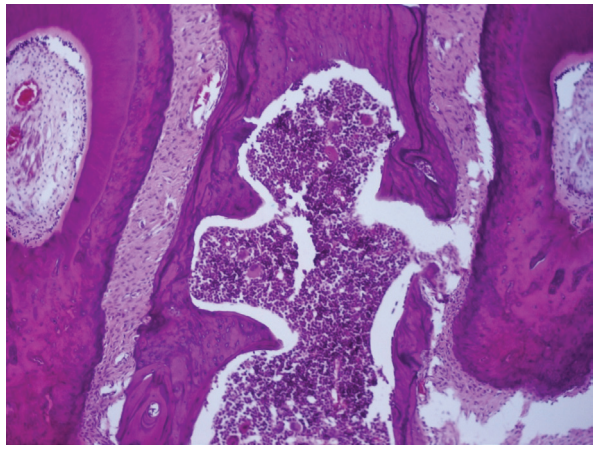

Figure 10. Alveolar bone in the interradicular area of a non-loaded first molar in an ovariectomized animal.

animals of Group A (control animals) (Figure 5).

Comparison of the measurements of orthodontic movement of the upper right first molars between Group A and Group B as calculated from the special dorsoventral radiographs, revealed statistically significant differences with higher values for the upper right first molars of the ovariectomized animals. Mean values and standard deviations of upper right molar movement in normal and ovariectomized groups are presented in Figure 6. The error for the tooth movement measurement was 0.03 mm and it was considered non-significant.

B. Histological findings

All tibias of the ovariectomized rats (Group B) were examined histologically in order to confirm systemic osteoporosis. It was observed that all tibias of the animals in Group B showed characteristic features of osteoporotic bone tissue. There was a disturbance in trabecular bone structure, revealed by trabecular separation, large marrow cavities (Figure 7) and little osteoblastic activity.

The alveolar bone in the apical region of the non-loaded upper left first molar of the ovariectomized animals (Group B) showed disturbed lamellar structure, obvious multiple cement lines and large areas of marrow cavities (Figure 8). In contrast, the alveolar bone in the apical area of the non-loaded upper left first molar of the control animals (Group A) showed lamellar dense bone with numerous osteoblasts and very little marrow cavities inbetween (Figure 9).

The alveolar bone in the interradicular area of the non-loaded upper left first molar of the ovariectomized animals

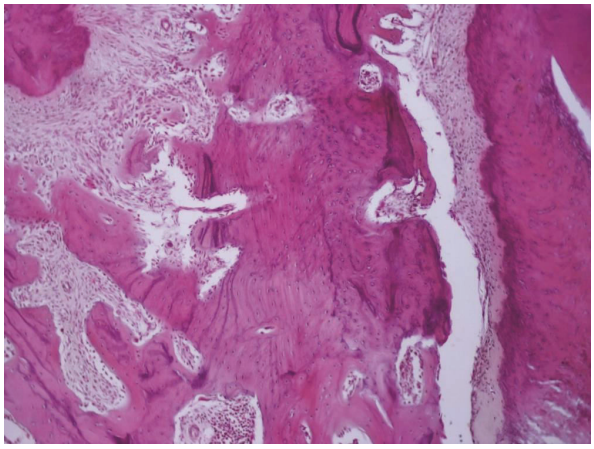

Figure 11. Alveolar bone in the interradicular area of a loaded first molar in an ovariectomized animal.

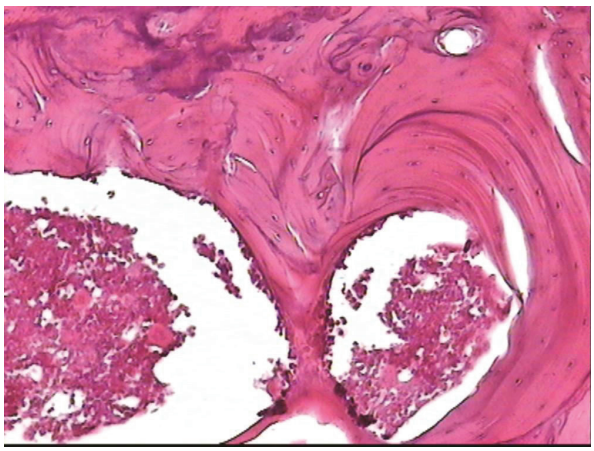

Figure 12. Irregular cement lines in alveolar bone of a loaded first molar in an ovariectomized animal.

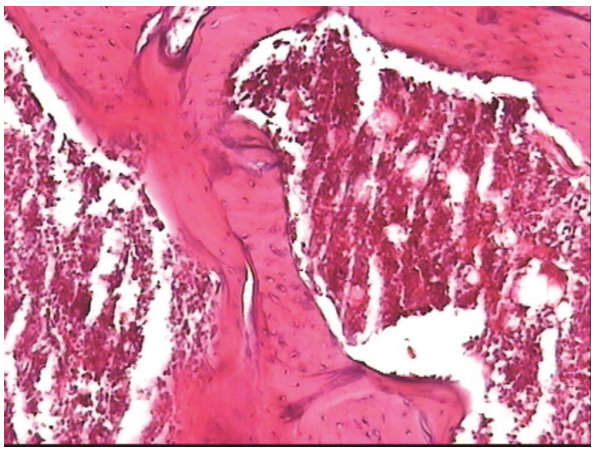

Figure 13. Numerous osteocytes embedded in the alveolar bone of a non-loaded first molar in a control animal.

showed multiple regular cement lines and extensive internal resorption resulting in large marrow cavities (Figure 10). In contrast, the alveolar bone in the interradicular area of the loaded upper right first molar of the ovariectomized animals revealed dense bone with almost no marrow cavity and frontal resorption on the surface (Figure 11).

In addition, the alveolar bone supporting the loaded upper right first molar in the ovariectomized animals revealed marked irregular cement lines and few osteocytes (Figure 12), as compared to the supporting alveolar bone of the non-loaded upper left first molar (Figure 13).

Discussion

The purpose of this experimental research study was to investigate in depth the effect of postmenopausal osteoporosis on the extent and quality of orthodontic tooth movement in mature female rats. The performance of bilateral ovariectomies in mature female Wistar rats 6 months of age was the method of choice for producing the experimental model of osteoporosis, or to be more accurate of osteopenia, as no fractures occur in this model of bone loss. Although it is well known that ovariectomy in mature female rats produces "osteoporotic" bone changes[15-17], histologic examination of the tibia of every ovariectomized rat was performed in order to confirm bone changes due to estrogen withdrawal.

The age of the experimental animals was one of our main concerns in planning our study protocol. In order to simulate results of postmenopausal osteoporosis, the animals should be no younger than 6 months of age. According to Jee and Yao[18], the female rat has reached the peak bone mass at the age of 9 months. Skeletal maturity in the rat happens later than sexual maturity. Previous experimental studies used animals of much younger age and they may have been describing results of orthodontic tooth movement due to estrogen loss in puberty or young adulthood[19-21]. It has been documented that the craniofacial complex in the rat still grows at the age of three months[22] and any postovariectomy effects are interweaved with skeletal growth effects. Postmenopausal osteoporosis in humans occurs during middle age, depends on the optimal bone growth during childhood and adolescence, and on the peak bone mass attained. Female rats aged 6 months or above are more appropriate models for the study of the orthodontic tooth movement in post-menopausal osteoporosis.

Orthodontic loading was applied on the upper first right molars of the experimental animals 60 days following ovariectomy. According to Wronski et al[23], the earliest time of bone loss in any part of the rat skeleton following ovariectomy is no less than 14 days, the earliest time of 50% bone loss in any skeletal part is no less than 30 to 60 days and the earliest time that a steady state in any bony part of the rat is achieved is no less than 90 days postovariectomy. Thus, a 60-day interval following ovariectomy is an efficient period of time for postovariectomy effects on bone to be expressed. From this standpoint, our experiment is in agreement with the

study of Arslan et al[20] and differs from the work of Yamashiro and Takano-Yamamoto[19], who used orthodontic forces 14 days after ovariectomy.

The closed coil spring method was selected for the mesial movement of the upper right first molars. By using this procedure, it is feasible to apply a fully controlled and reproducible amount of force, that may be measured with a dynamometer and, hence, study the pathophysiology and metabolism of the supporting alveolar bone in all tooth surfaces. On the contrary, the Waldo method[24], by using an elastic rubber band between the first and second upper molars, fails to cause movement for more than four days since the rubber band is removed from its position after the third day of placement[25]. The expansion spring described by Storey[26] and by Stark and Sinclair[27], modified by Arslan et al[20], may present stability and anchorage issues. Additionally, an appliance proposed by Ren et al[28] includes the maxillary molar unit as a whole and delivers the force to the whole molar unit instead of a single tooth.

The used force of 60 gr* is considered a heavy orthodontic force and it was purposely applied in order to influence both bone modeling and remodeling activities. According to Weijs and Dantuma[29], the rat molars are adapted to the exertion of large masticatory pressures. In the opinion of Ren et al[28], a 40 gr* force on a rat molar is comparable with a force of 2000 gr* on a human molar. It is difficult to agree with this statement, since even in the case that a human molar is 50 times larger than a rat molar, the special structural and myofunctional architecture of the rodent's stomatognathic system must be taken into account, as well as its mechanical demands. The overall masticatory forces are not dependent only on the tooth surface, but also on the capacity of the muscles of the stomatognathic system. The underlying alveolar and basal bone are accustomed to respective forces and therefore any relevant research with orthodontic forces applied in different species must take this into account.

Following the analysis of measurements of molar tooth movements, it was found that the average upper right first molar movement in the ovariectomized group was 45% greater compared to the average upper right first molar movement in the control group. This increase of tooth movement is similar to the one observed by Jin et al[30] in three-month-old Sprague-Dawley osteoporotic rats and also similar to the orthodontic movement of six-week-old Sprague-Dawley ovariectomized rats studied by Yamashiro and Takano-Yamamoto[19]. Also, Arslan et al[20] found similar results in their experimental study in ovariectomized three-month-old female Sprague-Dawley rats. Additionally Dai et al[21] reported a statistically significant increase in orthodontic tooth movement of their ovariectomized rats compared to the sham group.

According to Aschraft et al[31], the average molar movement of the animals subjected to cortisone injections that resulted in osteoporosis was three to four times greater, compared to the corresponding molar movement in the normal group. However, osteoporotic effects in bone tissue after cortisone intake are different from the osteoporotic bone changes due to estrogen withdrawal.

Furthermore, similar increase of orthodontic tooth movement has resulted in situations with high bone turnover as in hyperparathyroidism[32] and in hyperthyroidism[7]. On the contrary, the amount of tooth movement was reduced by approximately 50% in animals treated with receptors to IL-1, TNF-α and a combination of both[33]. Also, there was a 52.2% inhibition of tooth movement 14 days following force application in animals treated with OPG gene transfer to the periodontal tissues, compared to the tooth movement of the control animals[34]. On the other hand, local RANKL gene transfer into the periodontal tissue accelerated the amount of experimental tooth movement[35].

Administration of zoledronic acid[36] or alendronate sodium[37] decreased tooth movement in ovariectomized rats, but in both studies ovariectomy without therapy accelerated orthodontic tooth movement in a similar rate compared to our findings. Sirisoontorn et al[38] observed a higher rate of orthodontic tooth movement in the ovariectomized animals compared to our findings using lighter loading of the rat molars. Furthermore they concluded that ovariectomy affected not only tooth movement but also induced more severe root resorption compared to the control group. Comparing the higher rates of tooth movement in the previous work with our study's results using heavier movement forces, one may agree with the statement of Alikhami et al[39] that higher forces do not always result in increased tooth movement and that there is a "saturation in the biologic response" to orthodontic forces.

It has been found that there is a positive correlation between systemic osteoporosis and bone mineral density of the mandible[40]. An association between postmenopausal tooth loss and systemic bone loss has also been identified[41]. Furthermore, Anwar et al[42] demonstrated that estrogen deficiency results in porosity of the molar interradicular septum in ovariectomized monkeys and this microstructural damage was linked to their spinal osteoporosis. Tanaka et al[5] have also reported thin alveolar bone and osteoporotic changes of the trabecular bone in the interradicular septum of the rat first molar. Destruction of alveolar bone due to estrogen depletion was also found in ovariectomized sheep[43]. Estrogen deficiency induces osteoclastogenesis in the rat periodontium[44] and the resorptive localized activity induced by abnormal loading of the alveolar bone has been shown to be enhanced by this deficiency[45]. As a result of estrogen deficiency, a significant decrease in calcium levels in the rat mandible and incisors was observed after ovariectomy, correlated to the activity of the bone turnover markers in serum[46]. Regarding mandibular cortical thickness, there are studies that report its decrease[47], as well as no change[48] in ovariectomized rats compared to controls. Additionally, in the mandibular condyle of ovariectomized rats, cartilage thickness[49] and bone mass[50] are reported to be significantly decreased compared to control animals.

Our observation concerning the marked irregular cement lines found at the alveolar bone of the loaded molar in the ovariectomized animals can be attributed to the combined

action of loading and estrogen deficiency.

Our histologic results of the non-loaded side are in accordance with the findings of the prementioned investigations. The alveolar bone in the apical area of the first molar of the non-loaded side of the ovariectomized animals showed disturbed lamellar structure with large areas of marrow cavities indicating high bone turnover activity.

There is a lack of histological studies of orthodontic movement in osteoporotic animals. In the present study, the alveolar bone in the interradicular area of the loaded first molar in the ovariectomized group revealed frontal resorption, whereas the respective area of the non-loaded side showed extensive internal resorption with large marrow cavities. Thus, it appears that an osteoporotic alveolus with internal resorption due to estrogen deficiency may subsequently present frontal resorption due to tooth movement. These changes of alveolar histology after the application of local forces may be interpreted as a result of the effects of systemic osteoporosis following loading.

Conclusions

From our study it is concluded that osteoporosis due to ovariectomy influences the rate of orthodontic tooth movement, as well as the quality of alveolar bone remodeling in rats. An alveolus with osteoporotic internal resorption due to estrogen deficiency may be transformed, to some extent, to an alveolus with frontal resorption due to tooth movement. It seems that loading of the osteoporotic alveolar bone may alter the early effects of estrogen withdrawal on bone homeostasis.

Thus, clinicians must be aware of the increased rate of tooth movements in postmenopausal osteoporotic women undergoing orthodontic treatment, but also of possible improvement of the alveolus quality as a response to orthodontic loading. Further experimentation and documentation may elucidate the link between estrogen deficiency and tooth-loading effects in alveolar bone.

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