Alveolar bone response distal to applied orthodontic forces in ovariectomized rats

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Introduction

The application of forces on the teeth results in orthodontic tooth movement, as has been widely practiced during the last century. Consequently, there has been abundant documentation, both clinical and experimental1-5. Experimental research has shown that histological changes occur on a cellular level in the dentomaxillofacial system6. Following the loading of a tooth, alveolar bone formation occurs on the tension side of the root, and resorption is observed on the pressure side of the root2-5. Although this remodeling process is the basis of clinical orthodontic practice, the impact of applied forces on the teeth adjacent and distal to the loaded ones has not been thoroughly studied. Orthodontic tooth movement is also influenced by endocrine, paracrine and autocrine factors7,8. Estrogen deficiency in particular is associated with an increased rate of bone turnover and a leading cause of osteoporosis9,10. Apart from the known skeletal sites that postmenopausal osteoporosis affects, it has been observed that the jaws and alveolar bone are also influenced11,12. Ovariectomy and, therefore, osteoporosis in rats has been shown to significantly enhance the rate of tooth movement, as well as the rate of alveolar bone remodeling13-15. The present study was undertaken to

Abstract

Objectives: The aim of this study was to investigate the effect of the application of orthodontic tooth forces on the alveolar bone distal to the loaded teeth, in ovariectomized female rats. Methods: Twenty-four eight-month-old Wistar rats were divided into one group ovariectomized at the age of six months and one control. An orthodontic appliance delivering a mesial traction force of 60 gr* was placed on the right maxillary 1st molar of all animals for 14 days. Histology of the alveolar bone, of the adjacent and distal teeth to the loaded molar and the contralateral side, was performed following euthanasia. Results: In the non-ovariectomized rats, extensive resorption was noticed in the direction of the orthodontic movement in the 2nd and 3rd molar interdental space, whereas the respective contralateral interdental space did not show any remodeling activity. Ovariectomized rats displayed reduced osseous tissue in the interdental space of both sides. The alveolar bone in the interradicular area of the 2nd loaded molar revealed frontal resorption, whereas, the alveolar interradicular bone of the contralateral 2nd molar showed internal resorption. Conclusions: In conclusion, orthodontic forces applied to the dentoalveolar complex of ovariectomized rats affect bone remodeling, even in areas distal to the site of force application. This finding should be taken into account during orthodontic treatment of women during menopause.

Keywords: Orthodontic Forces, Orthodontic Tooth Movement, Osteoporosis, Ovariectomy, Rat

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examine the effect of the application of orthodontic forces on the alveolar bone distal to the loaded teeth, in normal and ovariectomized adult female rats.

Materials and Methods

a) Laboratory animals

Twenty-four six-month-old female Wistar rats were used in this study. The experimental animals were obtained from the Hellenic Pasteur Institute (Athens, Greece). The study received the permit no. K/3816/5-7-2005 from the Veterinary Directorate, according to the country’s legislation (Greek Presidential Decree 160/91), in conformation to the European Directive 86/609 regarding “the protection of vertebrate animals used in experimental and other scientific purposes”, which was in force at the time of the research study. The experiments took place in the Laboratory of Experimental Surgery and Surgical Research of the Medical School of the University of Athens and in the Orthodontic Department of the Dental School of the University of Athens. The rats were housed in a controlled environment, with a mean temperature 22±2 °C, relative humidity 55±5%, 15 air changes/hour, four to a cage, in polycarbonate cages (Tecniplast, Italy), with free access to tap water and standard pelleted rat diet (Mucedola, Italy).

b) Experimental procedures

The animals were divided into two groups consisting of twelve animals each, as follows: Group A included 12 rats that were subjected to orthodontic movement of the upper right first molars. Group B included 12 rats that were previously subjected to bilateral ovariectomy, followed by the same orthodontic movement of the upper right first molars.

Bilateral ovariectomies were performed in the female rats of Group B at six months of age. Under general anesthesia using intramuscular administration of ketamine hydrochloride 90 mg/kg and xylazine 5 mg/kg and aseptic conditions, ovariectomy was carried out from the ventral approach.

A split-mouth design was used, with the right side chosen as the experimental side and the left side serving as the control. Orthodontic molar movement was performed on all rats at 8 months of age (60 days post-ovariectomy of Group B) by the application of a closed coil spring (0.010 X 0.045 inches) extending from the upper right first molar to the upper right central incisor. The coil spring was 1 cm in length, and its activation for 0.25 cm produced a force of 60 gr*. The orthodontic force was applied for 14 days, during which the rats had access to their diet in both pelleted and pulverized form. At the end of this period, following euthanasia of the animals, the spring was carefully removed and the upper jaw dissected from the skull. The animals were observed daily and their clinical appearance, body weight and food consumption were registered.

c) Histology

The specimens were fixed in 10% buffered formalin for 18 hours and decalcified in EDTA buffer for 6-8 weeks. The apical regions were dissected sagittally to 5 mm-thick slices including the central incisor to the third molar area. The specimens were dehydrated with ethanol and embedded in paraffin. Histological sections, 4 to 5 μm-thick, were obtained from each specimen, stained with Hematoxylin and Eosin, and were observed under transmitted light microscopy.

Institutional Review Board Statement

The study was conducted according to the country’s legislation (Greek Presidential Decree 160/91), in conformation to the European Directive 86/609 regarding “the protection of vertebrate animals used in experimental and other scientific purposes”, which was in force at the time
of the research study. It received the permit no. Κ/3816/date 5-7-2005 from the Athens Veterinary Directorate, Greece.

Results

a. Clinical findings of the laboratory animals

All animals survived the operations of ovariectomy and orthodontic force application. A non-significant transient reduced food consumption and body weight loss was noticed in most animals during the first days of force application. Oedema of the gingival was observed postoperatively after application of the spring in 30% of the animals, which regressed 3 days later. Clinical symptoms of inflammation were not observed.

b.1 Histological findings in the non-ovariectomized rats

- On the right side of the maxilla, where the orthodontic forces were applied, the alveolar bone in the interdental space between the 2nd and 3rd molars revealed remodeling activity. Extensive resorption was noticed in the direction of movement, whereas bone formation appeared on the rear side of the interdental space and was less prominent. The osseous tissue of the interradicular area of the 2nd molar showed internal resorption with preservation of the alveolar bone architecture (Figures 1a, 1b).
- On the left side of the non-ovariectomized rat maxilla, where no orthodontic forces were applied, the alveolar bone in the interdental space between the 2nd and 3rd molars was denser and did not show any activity. Nevertheless, the osseous tissue of the interradicular area of the 2nd molar also revealed internal resorption (Figures 2a, 2b).

b.2 Histological findings in the ovariectomized rats

- On the right side of the maxilla, where the orthodontic forces were applied, the alveolar bone in the interdental space between the 2nd and 3rd molars was dense, but in
comparison to the non-ovariectomized rats, the amount of osseous tissue was reduced, and replaced by fibrous tissue with newly formed vessels (Figures 3a and 3b). The osseous tissue of the interradicular area of the 2nd molar was also dense (Figures 3c), but was markedly decreased in comparison to the non-ovariectomized rats. The interradicular alveolar bone showed resorption on the surface facing the proximal root, whereas apposition was displayed distally. The periodontal ligament (stroma) surrounding the interradicular area was broader compared to that of the non-ovariectomized rats, and fibrotic, with extensive angiogenesis.

- On the left side of the maxilla where no orthodontic forces were applied, the alveolar bone in the interdental space between the 2nd and 3rd molars showed minor remodeling activity (both resorption and formation) but the amount of the osseous tissue was markedly decreased compared to the non-ovariectomized rats (Figures 4a and 4b). Additionally, the osseous tissue of the interradicular area of the 2nd molar showed marked remodeling activity causing extensive internal resorption, expressed with increased porosity of the alveolar bone (Figures 4c). The behavior of the bone in this region was similar to that of the non-ovariectomized rats.

**Discussion**

The present study aimed at detecting possible bone changes caused by orthodontic forces distal to their application, both in normal, as well as in osteoporotic rats. The effect of orthodontic forces on normal bone adjacent to their application has been well documented both in humans\(^9,20\) and in the ovariectomized rat model\(^{21\text{-}23}\). However, there is limited information on the effect of loading on osteoporotic bone. In corticosteroid-induced osteoporotic New Zealand White rabbits aged 3 months old, Ashcraft et al. have demonstrated an increase in the rate of orthodontic tooth movement\(^{24}\). Takano-Yamamoto have reported that ovariectomy in 2-month-old rats accelerated orthodontic tooth movement, as shown by histomorphometry\(^{25}\). In addition, Tsolakis has also shown that ovariectomy-induced osteoporosis in rats increases the rate of orthodontic tooth movement, as well as it affects the quality of alveolar bone remodeling\(^3\). Recent studies have demonstrated by micro-CT that tooth movement was greater in the ovariectomized rats compared to the non-ovariectomized animals\(^{26\text{-}27}\). These studies have focused on the changes induced on the implemented tooth and its surrounding tissues. To our knowledge, based on the available literature, changes occurring to the neighboring and distal teeth and their supportive bone, have not been studied. The present study examined this area and the changes therein.

Our study was carried out on mature female rats. According to Jee and Yao, the optimal age for an ovariectomized rat to simulate post-menopausal osteoporosis is 9 months\(^9\). At this age, they have achieved peak bone mass. In our experiment, ovariectomy was performed at 6 months of age and orthodontic forces were applied when they became 8 months old, for a period of 2 weeks, which is the appropriate experimental period advised by previous and recent studies\(^{28\text{-}29}\). This consists a unique procedure in comparison with other studies, which have conducted ovariectomy in younger rats\(^{22\text{-}25\text{-}27\text{-}30}\).

In the present study, a split-mouth design was chosen to take into account variable alveolar compensatory remodeling effects, due to the physiological distal drift of the rat molars\(^2\text{-}28\). Also, the split-mouth design limited the inter- animal variation due to the effect of osteoporosis.
The applied force of 60 gr* is considered as a heavy orthodontic force and it was purposely designed in order to influence both bone modeling and remodeling activities. According to Ren et al., a 40 gr* force on a rat molar is comparable to 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. Weij and Dantuma state that the molar topography of the rat may be explained as an adaptation for exerting large masticatory pressures. The underlying alveolar and basal bone are accustomed to respective forces and therefore any relevant research with orthodontic loading applied in different species must take this into account. Specifically, in the rat, Alikhani et al. demonstrated that forces in excess of a certain magnitude, i.e. above 10 gr*, and up to 100 gr*, produce similar tooth movement. In agreement with King et al. who considered that a force of 20-40 gr* is necessary for tooth movement, a recent orthodontic study in rats used a 30 gr* spring force; however it was on male rats with nicotine effects as an outcome.

According to Frost, bone strength index as well as the minimum effective strain, vary for the same organs in different species and the strain magnitudes in skeletal organs depend on the relationship between their strength and the size of the loads on them. Taking into account the above mentioned feature of the Utah paradigm, the different anatomic and functional characteristics of the two species, human and rat, as well as the different forces generated from the masseter muscles and applied to the molar teeth of the two species, a force of 60 gr* applied to the rat molar may be comparable to a force of 480 gr* applied to the human molar. These forces may be compared to the orthopaedic forces applied to human molar teeth through application of extraoral appliances.

An interesting finding was the frontal resorption noticed in the interradicular area of the 2nd molar of the orthodontically loaded side in the ovariectomized group of rats, in contrast to the internal resorption observed in the interradicular area of the second molar in the contralateral side of the non-ovariectomized group. This remodeling process is similar to the one observed in the alveolar bone of the inter-radicular area of the upper right first molar that was subjected to the orthodontic force of 60 gr*. It seems that there is a reversal pattern of ovariectomy-induced osteoporotic bone remodeling, due to the loading of the alveolar bone. This is an interesting finding as regards the biology of osteoporotic bone in general, since it is possible to change the osteoporotic remodeling process by the application of mechanical loading.

In the biology of tooth movement, the pressure and tension hypothesis strictly related to the periodontal – ligament area has been used to explain changes around the orthodontically moved tooth. A recent micro-CT study has demonstrated that light orthodontic forces (10 gr*) induce bone resorption on the compression side and bone formation on the tension side of the affected tooth. Although it is thought that forces applied to individual teeth may affect the alveolar bone of the proximal teeth, as well as the relative cortical bone, there has been no attempt to study possible changes in neighboring areas to the loaded ones. Additional recent studies have suggested that when a bone is loaded, osteocytes sense it through the interstitial bone fluid in the canaliculae and osteocyte lacunae, and generate signals that may influence cells near or on a distant bone surface. Transmission of the afore-mentioned signals is through the functional syncytium of osteocytes, bone lining cells and stromal cells, and the gap junctions and hemichannels between them, which play important roles in the regulation of mechano-transduction in bone. This syncytium of cells that detects and transmits information is considered to play a role in the control of modeling and remodeling. Hence, strains on a certain bone area may influence neighboring structures of the same bone.

Although it is generally observed that orthodontic tooth movement is increased after oestrogen depletion in animals, an interesting finding in a clinical study demonstrated no significant changes in the bone biomarkers RANKL and osteopontin locally (in the gingival crevicular fluid) one day following orthodontic application of forces between pre- and post-menopausal women. Further studies examining these biomarkers one and two weeks after orthodontic activation would be enlightening regarding their long-term production and their correlation to orthodontic movement and alveolar bone turnover.

Conclusions

Our findings support the conclusion that changes in the alveolar bone are also observed in areas distal and remote to the mechanically loaded ones, and confirm that bone bending and remodeling of the periodontal tissues are interrelated in orthodontic tooth movement. These findings should be taken into account during orthodontic treatment of women during menopause.

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