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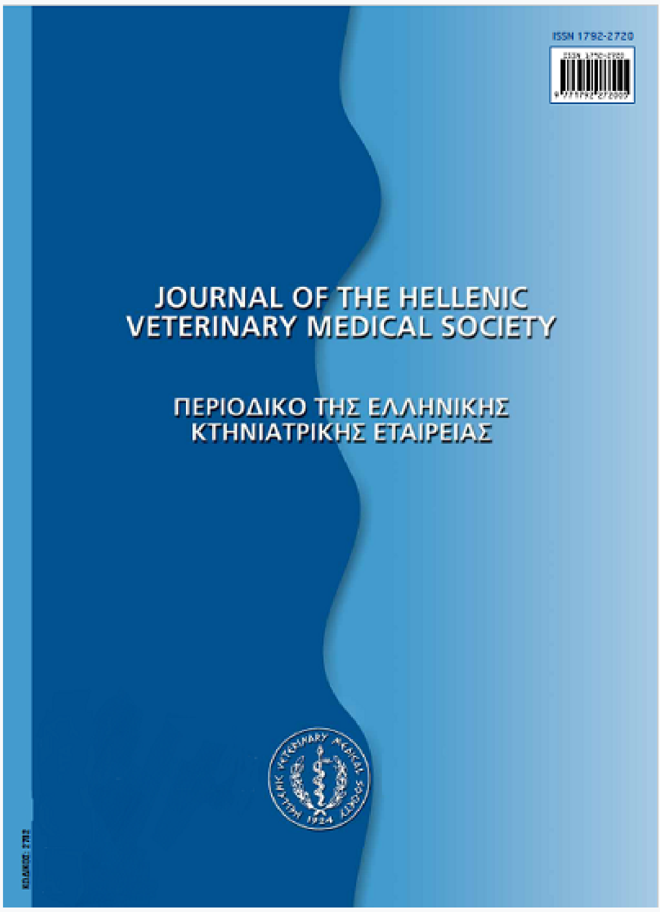

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Regional Acceleratory Phenomenon after Orthodontic Force Exertion in Ovariectomized Rats

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ABSTRACT. The application of orthodontic forces may be one of the factors that produce a regional acceleratory phenomenon (RAP) in mandibular and maxillary bones. The effect of exerted forces on bone tissue ahead of their point of application has not been extensively studied. Moreover, limited information exists regarding this phenomenon on osteoporotic bone. The study aim was to examine the role of orthodontic forces on the expression of RAP in normal and osteoporotic mature rats. Thirty-six eight-month-old skeletally mature female Wistar rats, half of which had been previously ovariectomized (OVX) at the age of 6 months, were subjected to orthodontic movement of the upper right first molar. An orthodontic force of 60 gr* was generated through a closed coil spring for 14 days. The maxillae were then removed and the area ahead of the first molar was examined histologically. On the side of orthodontic force application, distortion of bone structure and woven bone formation were observed in non-OVX rats, whereas in the OVX rats, extensive remodeling was apparent. In conclusion, the application of orthodontic forces on both normal and osteoporotic mature rats in the present study created a RAP ahead of the loaded teeth demonstrated histologically, indicating increased bone resorption and formation in the OVX rats.

Keywords: regional acceleratory phenomenon (RAP), orthodontic forces, osteoporosis, ovariectomy, rat

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INTRODUCTION

Orthodontic forces have been applied on growing and adult humans, in order to treat dentofacial abnormalities during the last decades. Research has focused on the tissues and the cellular changes surrounding the loaded teeth and their corresponding periodontal ligaments (Spyropoulos, 2008). Additionally, experimental studies have been conducted on normal and ovariectomized rats regarding the effects of orthodontic forces on tooth movement, root resorption, and both cortical and alveolar bone changes around the instrumented tooth (Tsolakis et al., 2008; Sirisoontorn et al., 2011; Sirisoontorn et al., 2012; Ru et al., 2013). However, there is limited information concerning the bone areas ahead to the application of forces.

A regional acceleratory phenomenon (RAP) may be produced by orthodontic forces, traumatic and stress fractures, lacerations, as well as by infectious and non-infectious inflammations (Frost, 1983; Frost, 2004). During a RAP, all ongoing regional processes are accelerated and it seems to represent an "SOS" mechanism during serious noxious stimuli (Jee and Yao, 2001). Moreover, RAPs normally improve the body's ability to resist and manage established infections, as well as the resulting healing in all hard and soft tissues (Frost, 2004).

Recent concepts interrelating bone biology and skeletal bio-mechanics consider microdamages of bone tissue as events that initiate bone modeling and remodeling (Parfitt, 2002; Martin, 2003). Moreover, it seems that the initial periodontal ligament response to orthodontic loading is related to hypertrophic and fatigue mechanisms (Katona et al., 1995; Roberts, 2000.). Furthermore, Verna et al suggest a role for microcracks in the initiation of bone remodeling following the application of orthodontic forces (Verna, 2004).

We therefore hypothesize that if orthodontic forces exerted on bone are perceived as traumatic or non-infectious stimuli by the local tissues, followed by microdamage, RAP phenomena may potentially appear in the surrounding bone tissue. The ovariectomized rat model of postmenopausal osteoporosis is an established model of skeletal osteopenia and is chosen for the present experimental study (Lelovas et al., 2008). Thus, the purpose of our study is to determine any RAP phenomena ahead of the application of heavy orthodontic forces on the molars of mature rats, both on osteoporotic and intact animals.

MATERIALS AND METHODS

<u>Laboratory animals</u>

Thirty-six eight-month-old skeletally mature female Wistar rats were used in this study, with a mean body weight of 310±20 g (mean±SD). The study protocol was approved by the Veterinary Directorate, according to the national legislation (PD 160/1991) which was in force at the time of the study and which was conformed to the European Directive 86/609 (license no. K/3816/26.10.05). The animals were housed in the Laboratory of Experimental Surgery and Surgical Research, School of Medicine, National & Kapodistrian University of Athens, Greece. They were housed two to a cage in conventional open-top cages, in environmentally controlled light conditions (12/12 hours light/dark), temperature range 22±2 °C, relative humidity 55±5 % and 12 air changes/hour, with unlimited access to standard pelleted rodent diet (Mucedola 4RF18) and tap water. The animals were divided into two different groups consisting of eighteen animals each, as follows: Group A included 18 rats that were subjected to orthodontic movement of the upper right first molars without any surgery. Group B included 18 rats that were subjected to orthodontic movement of the upper right first molars after bilateral ovariectomy which was conducted 2 months previously.

<u>Ovariectomy</u>

Bilateral ovariectomies were performed in the Group B female rats from the ventral approach at the age of 6 months, according to the model of postmenopausal osteoporosis. Briefly, the animals were weighed and anesthetized by i.m. injection of ketamine hydrochloride 100 mg/kg b.w. (Ketaset, Pfizer) and xylazine 5 mg/kg b.w. (Rompun, Bayer) for surgery. Following loss of consciousness, chemoprophylaxis with enrofloxacin 10 mg/kg b.w. (Baytril 5%, Bayer) and pre-emptive analgesia with carprofen 0.08 mg/kg b.w. (Rimadyl, Pfizer) were administered subcutaneously. Using aseptic procedures, a midline ventral incision through the linea alba was performed, the ovaries dissected, ligated and removed bilaterally, and the peritoneum and skin were closed in layers using single interrupted sutures. The operation was performed on a heating pad to prevent hypothermia. Recovery from anesthesia was monitored in a heated recovery cage until the rats were mobile, following which they were returned to their respective cages. Chemoprophylaxis and analgesia were repeated for three post-operative days and the wound closure monitored daily for 10 days, after which the sutures were removed.

Application of orthodontic forces

Orthodontic rat molar movement was achieved 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 nickel-titanium coil spring was 1 cm in length, and its activation for 0.25 cm produced a force of 60 gr*, that was measured with the Haldex precision force gauge. The orthodontic force was applied for 14 days. At the end of the experimental period, following euthanasia of the animals, the maxillary bone was dissected from the skull and the spring was removed carefully.

Histology

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 sagittally, including the cortical maxillary bone region from the central incisor to the first molar. The specimens were then dehydrated with ethanol and embedded in paraffin. Histological sections, 4 to 5 μm-thick, were obtained, stained with hematoxylin and eosin and were observed under transmitted light microscopy.

Quantitative estimation of the quality of the cortex was achieved using a scoring system as following: 0=no changes, 1=mild changes, 2=moderate changes and 3=severe changes. Mean values were determined for the final score. The cortex thickness was measured using a micrometer attached to the eyepiece of the microscope.

RESULTS

Laboratory animals

All animals survived the operations of ovariectomy and orthodontic force application uneventfully. A non-significant transient body weight loss, ranging between 2 and 7 g per rat (less than 3% b.w.), in spite of the administration of powdered food (grinded pellets), was noticed in most animals during the first week of force application. This was attributed to the initial discomfort of the application and was followed by recovery of previous body weight in the next week.

Histological findings of non-ovariectomized rats

The cortical bone of the non-OVX rats, on the right side and ahead of the right first molar where the orthodontic forces were applied, showed distortion of

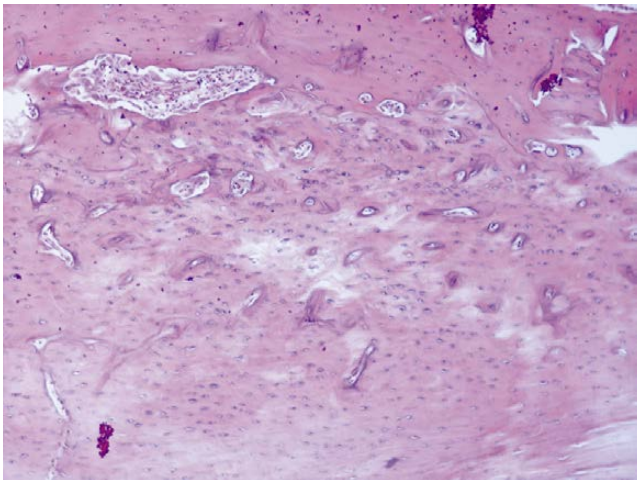

Fig 1. Cortical bone of non-ovariectomized rats, on the right side ahead of the 1st upper molar with force application, showing marked distortion of bone structure and woven bone (arrow) formation (Obj. X10 H-E).

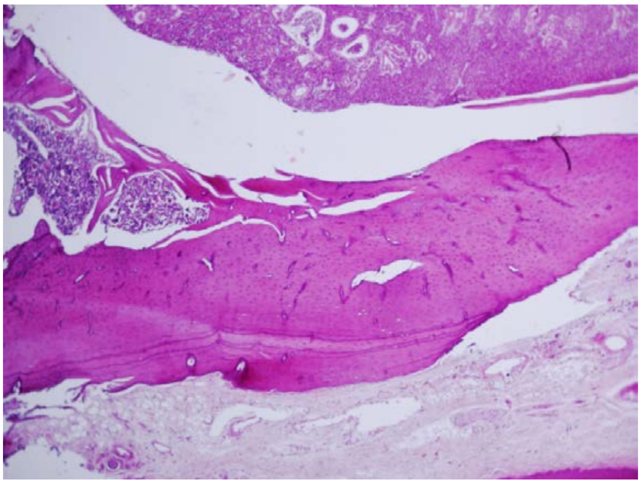

Fig 2. Cortical bone of non-ovariectomized rats, on the left side ahead of the 1st upper molar without any force application, where the bone is lamellar (arrow) and well oriented (Obj. X4 H-E).

bone structure and woven bone formation (Figure 1). The final score showed moderate changes=2.

In contrast, the left side, where no orthodontic forces were applied, the bone ahead of the left first molar was lamellar and well oriented (Figure 2) with a final score of mild changes=1.

Histological findings of ovariectomized rats

The cortical bone of the OVX rats, on the right side and ahead of the right first molar where the orthodontic forces were applied, showed distinct cement lines.

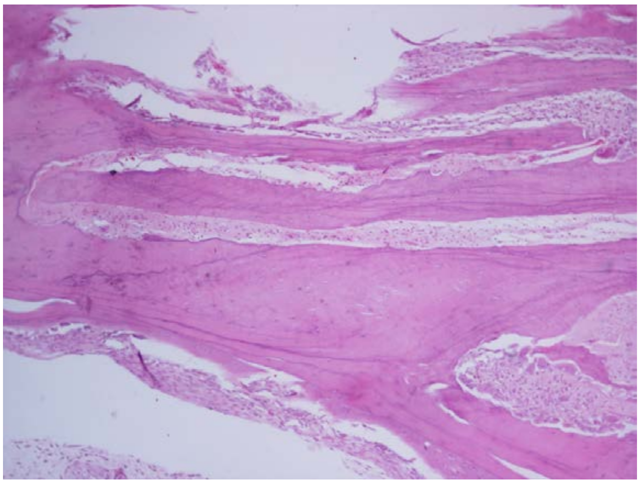

Fig 3. Cortical bone of ovariectomized rats, on the right side ahead of the 1st upper molar with force application, showing distinct cement lines (arrow), with increased bone resorption (arrowhead) and formation (white arrow) (Obj. X10 H-E).

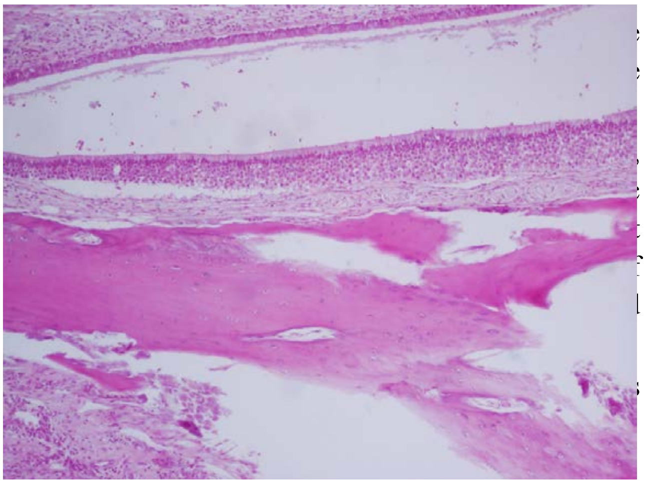

Fig 4. Cortical bone of ovariectomized rats, on the left side ahead of the 1st upper molar without any force application, showing a more compact structure (arrow) compared to the right side (Obj. X10 H-E).

researchers in the field of tooth movement concentrated their attention on the biologic changes mainly on the alveolus surrounding the displaced teeth (Reitan, 1951; Rygh, 1972; Davidovitch, 1991; Brudvik and Rygh, 1994; Masella and Meister, 2006; Meikle, 2006). Recent experimental work with orthodontic tooth movement in dogs has documented RAP in alveolar bone with histomorphometry (Deguchi et al., 2008).

The RAP is considered to increase regional or local bone remodeling, in response to an irritating intervention, such as, in the present case, force application (Jee and Yao, 2001; Verna, 2016). The RAP's severity depends on the intensity of the stimulus that has turned it on (Frost, 2004). In the present study, the magnitude of the applied orthodontic force was high (60 g*). The RAP was microscopically apparent at the cortical bone region between the central incisors and the 1st molar, two weeks following the application of the orthodontic loading.

Bone is a complex, living tissue that possesses the ability of constant adaptation to structural and metabolic demands. An interconnection and interrelationship of the osteons exists through the tubular canaliculae and therefore any stimulus on the external bone surface is distributed to the inner bone. Tubular canaliculae help osteocytes monitor and detect local bone stresses, strains and microdamages (Marotti, 2000). Furthermore, osteocytes communicate with each other and with bone lining cells via gap junctions (Jiang et al., 2007). In this sense, any change in tooth loading is distributed by osteocytes not only to the alveolar bone, but also to the entire surrounding bone tissue.

The effect of tooth loading in adjacent tissues in normal rats has been studied extensively, as mentioned above. However, there has not been systematic investigation of bone tissue changes ahead of the loaded tooth. Melsen (2001) observed woven bone formation ahead of the alveolus in the direction of the displacement in normal rats, and interpreted it as a RAP. In our study, the application of forces evoked accelerated changes, as shown by histological sections in both normal and osteoporotic mature animals. It has also been shown in dogs that there is accelerated tooth movement due to a regional acceleratory phenomenon following corticotomy-facilitat-

	Force application	No force application
OVX rats	3	1
Non-OVX rats	2	1

Table legend. Table indicating the cortical bone histological changes expressed with mean values of each rat group as calculated by the following scoring system:

1= Mild changes

2= Moderate changes

3= Severe changes

ed technique (Mostafa et al., 2009). Given the fact that the appropriate age of the female rat model for post-menopausal osteoporosis should be no less than 6 months, the rats of the present study were ovariectomized at that age. Additionally, as the earliest time of bone loss in different skeletal rat sites is noted between 14 and 60 days (Jee and Yao, 2001), we applied the orthodontic forces at the end of that period.

It seems that the strains exerted on the surrounding bone tissues ahead of the loaded teeth affected their modeling and remodeling processes. The effect of OVX on rat cortical bone ahead of the first molar was expressed by an increase of bone remodeling, whereas the effect of force application appeared to affect the structure of the bone.

The combination of OVX and retraction caused catabolic (resorption) and anabolic (increase in thickness) modeling of bone additionally to the structural distortion (woven bone). Our findings at the site ahead of the loaded tooth are similar to the tissue changes that Roberts et al. demonstrated adjacent to the loaded tooth, as initial responses to tooth movement (2004). According to their study, catabolic and anabolic modeling occurs at the periodontal ligament of the loaded tooth. The findings of this experimental study may have significant clinical value in cases where orthodontic treatment of mature adults is warranted, in particular of postmenopausal women who suffer from systemic osteoporosis. The potentially increased mandibular and/or maxillary bone resorption and formation in these patients, in response to orthodontic forces applied, may necessitate modification of the normal adult treatment period and magnitude of applied forces.

CONCLUSION

The application of orthodontic tooth forces on both normal and osteoporotic mature rats, in the present study, created a regional acceleratory phenomenon ahead of the loaded teeth, which was demonstrated histologically. In the normal rats the RAP was expressed with distortion of bone structure and the presence of woven bone, whereas in the osteoporotic rats the RAP was expressed with increased catabolic, as well as anabolic remodeling. These experimental findings should be taken into account in clinical practice when orthodontic forces are applied to mature patients with osteoporosis.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest

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