The Effects of Ovariectomy and Naproxen Treatment on the Strength of Femoral Midshaft and Molar Alveolar Region in Rats

Efectos de la Ovariectomía y Tratamiento con Naproxeno sobre la Resistencia de la Parte Media de la Diáfisis Femoral y la Región Alveolar Molar en Ratas

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SUMMARY: The goal of the present study was to investigate the effects of ovariectomy and naproxen treatment on both femoral and mandibular bone mass and biomechanical competence. Sprague-Dawley rats were used and divided into five groups: baseline, sham ovariectomized, ovariectomized, sham ovariectomized + naproxen treatment, ovariectomized + naproxen treatment. Mandibles and femurs of the rats were extracted and bone mineral density of the extracted specimens were determined. The volumes and ash weights of the femurs and mandibles were estimated. Maximum loads of the femurs and mandibles were determined by using a three point bending test. Ovariectomy decreased bone mineral density of the femoral midshaft, however naproxen prevented this decrease. Neither ovariectomy nor naproxen treatment affected the BMD in the molar alveolar region. Maximum load was found significantly decreased in the femoral midshaft, however, naproxen treatment prevented this decrease. Maximum load of the molar alveolar region did not significantly change. Naproxen prevents the strength characteristics of femoral midshaft afforded by ovariectomy. However, neither ovariectomy nor naproxen has an effect on the molar alveolar region of the mandible.

KEY WORDS: Ovariectomy; Naproxen; Bone mineral density; Maximum load.

INTRODUCTION

The ovariectomized rat as a model for human postmenopausal osteoporosis has been frequently used to demonstrate the alterations of mass, structure, and the mechanical properties of bone in the vertebra, femur and tibia (Frost & Lee, 1992; Jiang et al., 2007; Zhang et al., 2007), but a few conflicting reports have evaluated the changes in the properties of mandible (Ortman et al., 1989; Jeffcoat, 1998; Yang et al., 2005).

Naproxen (N), one of the non-selective NSAIDs, which are commonly used in inflammatory diseases, such as rhematoid arthritis and periodontitis (Vane & Botting, 1995), was found that it depresses the activation frequency, that prevents the structural and massive degeneration in the tibias of old immobilized rats (Lane et al., 1994), that blocks the bone resorption in the tibias (Lane et al., 1990) and that partially prevents the bone loss in the femurs and vertebraes of ovariectomized rats (Lane et al., 1990; Jiang et al., 1998). A few studies reported that N inhibited alveolar bone loss in periodontitis (Howell et al., 1991) and that furthermore provided new bone gain (Jeffcoat et al., 1991). On the other hand, it was shown that N treatment improved the vertebral strength (Jiang et al., 1998). We therefore aimed to investigate the effects of ovariectomy (OVX) and naproxen treatment on both femoral and mandibular bone mass and biomechanical competence.

MATERIAL AND METHOD

Sixty female Sprague-Dawley rats of 24 weeks old
were used in this study. Initial body weights of rats were 260 (SD± 17) g. Throughout the experiment, the rats were kept under the following conditions: room temperature, 20° ±1°C, humidity 50% ±20%, and lights on for 12 h/ day (800–2000). All rats were allowed free access to water and a pelleted commercial diet (containing 0.97% calcium, 0.85% phosphorus and 1.05 IU/g of Vitamin D3). After a week of acclimation, animals were used for studies. The researchers had their certificates (Use of Laboratory Animals in Biomedical Research) of the University and every effort was made to minimize both the number of animals and their suffering.

Animals were randomly divided into five groups of 12 rats each, as follows: (1) Baseline, (2) Sham ovariectomized (SHAM), (3) OVX, (4) SHAM+N treatment, (5) OVX+N treatment. The baseline group was euthanized by sodium pentobarbital overdose (Nembutal, Abbott Laboratories, Chicago, IL, USA) at the day the others were ovariectomized or sham operated. In order to induce systemic osteopenia, OVX groups were bilaterally ovariectomized with a dorsal approach under i.p. sodium pentobarbital (40 mg/kg body weight) anaesthesia. Success of ovariectomy was confirmed at necropsy by failure to detect ovarian tissue and by observation of marked atrophy of uterine horns. Same surgical procedure except the removal of ovaries was performed on the rats in the SHAM groups. Naproxen was given for 10 weeks, by mixing 10 mg/kg body weight per day to the food.

The mandibles and femurs were extracted and the soft tissues were removed following euthanization. Mandibles were separated at the symphysis into two halves. The bones were wrapped in saline-soaked gauze, stored at -20°C in closed tubes, following the measurements of bone volume and bone mineral density (BMD).

The volumes of the femurs and hemimandibles were estimated by weighing (Mettler AG 245, Switzerland) the bone specimens before and during immersion in water. The femurs and hemimandibles were subsequently scanned using bone densitometer (Hologic QDR 2000 DXA, Waltham, MA, USA) to measure their BMD (mg/cm²) using the small animal software.

Each hemimandible was positioned with the lingual side up and the femur proximal side up. The specimens were placed in a recipient with saline solution. Femur and hemimandible BMD measurements were made from the midshaft and the lower part of the molar alveolar region (AR), respectively. For the femur, the midpoint of ROI (region of interest) was determined as the midpoint of the distance between the greater trochanter and the lateral condyle in midshaft, and the midpoint of the distance between the tip of the incisor and the condylar process in the hemimandible. Five consecutive measurements of the regions of the femurs and hemimandibles were estimated.

Maximum loads (Lmax, a measure of the force that the bone fracture occurred) of the femurs and hemimandibles were determined by using a three point bending test. Each femur was placed on the two supports (spaced 15 mm apart) of the materials testing machine (LRX, Lloyd Instruments Plc, Fareham, Hampshire, England), with the medial side facing upwards. Due care was taken to ensure one of the support was placed under the greater trochanter and the other under the distal femur. The loading point was determined as the midpoint of the distance between the greater trochanter and the lateral condyle in the femoral midshaft. This point was exactly at the middle of the distance between the two supports. The force was applied with a rod (at a constant deformation rate of 1 mm/min) directly to the femoral midshaft until the sample fractured. Strength measurements in hemimandibles were detected by measuring Lmax with a three-point bending test. The midpoint of the distance between the tip of the incisor and the condylar process on the buccal side of each mandible was determined as the central loading point. Paying attention to locate this point centrally between the supports set 10 mm apart, the mandible was placed in a way to face the lingual surface down side. To ensure better distribution of the load on the irregular bottom surface of the mandible, sponges were placed between the outer bottom surface of the mandible and supports. Load was applied at a deformation rate of 1 mm/min until the sample fractured.

After biomechanical testing, the fractured rat femurs and hemimandibles were burned at 600°C, for 24 h and ash weight was measured and the ash content was expressed as mg/mm³ of specimen volume (ash density=ash weight/total tissue volume).

Statistical analysis was done using the SPSS 16.0 for Windows software package. Preliminary analyses of the data revealed that several measurements were not normally distributed and therefore non-parametric tests were used. When values of some groups were different according to the Kruskal-Wallis test, the Mann-Whitney U test with Bonferroni correction was used to determine which differences of each two groups were significant. A p value of less than 0.005 was considered significant. Data correlation was assessed using Spearman's rho.
RESULTS

Body weight increased (42%) in SHAM rats (p<0.001), 10 weeks after the operation, as a function of aging. Ovariectomized rats weighed (23%) more than SHAM rats (p<0.001). No significant difference was found in body weights between SHAM and SHAM+N, and between OVX and OVX+N (Fig. 1).

BMD in the femoral midshaft increased significantly in the SHAM group 10 weeks after the operation when compared to baseline (p<0.002), while there was a significant decrease in the OVX group when compared to the SHAM (p<0.005). No difference was found in BMD between SHAM and SHAM+N whereas BMD in the OVX+N group was significantly higher than the OVX group (p<0.005). OVX did decrease BMD of the femoral midshaft, and N prevented this decrease (Fig. 2).

![Fig. 1. Body weights of the rats.](image1)

![Fig. 2. BMDs of femoral midshafts.](image2)
Neither OVX nor N treatment affected the BMD in the AR. No significant difference existed between SHAM and OVX, SHAM and SHAM+N, and also OVX and OVX+N groups (Fig. 3).

Lmax of the femoral midshaft decreased in the OVX group when compared to SHAM (p<0.002), whereas OVX+N group indicated a significantly higher Lmax than the OVX group (p<0.001) (Fig. 4). However, these groups did not reveal any significant differences when Lmax of the AR was estimated (Fig. 5). There were no significant differences between the groups either in femoral and mandibular total volume or ash density. The only significant correlation existed between BMD and maximum load in the femur (r=0.320, p<0.05).

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Fig. 3. BMDs of the molar alveolar regions in the mandibles.

Fig. 4. Maximum loads of the femoral midshafts.
DISCUSSION

Our study showed that the effects of OVX and N treatment on the femoral midshaft and molar alveolar region of the mandible were different. OVX was found to decrease BMD and Lmax in femoral midshaft but not in the AR, 10 weeks after the operation. N treatment did prevent the decrease in the BMD and the Lmax in the femoral midshaft induced by ovariectomy, while it had no effect on the BMD and Lmax of the AR.

All of the rats gained weight during the course of the study. The body weight of the ovariectomized rats were significantly increased when compared to SHAM rats, at 10 weeks post-operation. Studies have shown that the removal of estrogen is related to an increase of food intake and weight gain in rodents (Chen & Heiman, 2001). Treatment with naproxen did not affect either aging or OVX related body weight gain, that agrees with the findings of some previous reports (Jiang et al., 1998; Solheim et al., 1986).

10 weeks after the operations, BMD and Lmax in the femoral midshaft which is mostly formed of cortical bone was found to be increased in all groups when compared to baseline, whereas they decreased in ovariectomized rats when compared to SHAM rats. These findings partially agrees with the researches which age of rats and post-ovariectomy periods were similar in study design. In these studies, the reason why BMD increases with regard to baseline in femoral midshaft in all groups within 12 weeks following ovariectomy and starts to decrease after 12 weeks (Jiang et al., 1998; Jiang et al., 1997) may be the use of young (18 week) rats which skeletal development is faster. In a study, it is reported that BMD and Lmax in femoral midshaft decrease after 12 weeks following OVX in 32 week rats (Akhter et al., 2003), while no significant change is determined in femoral midshaft of 12 week rats in another research (Zhang et al.). These different results may have been caused by different levels of osteopenia due to difference in the age of the animals at OVX and different post-ovariectomy periods (Patlas et al., 2000).

Cortical bone is not very sensitive to bone loss due to ovariectomy in spite of increased endosteal osteblasts (Liu et al., 1990; Jee et al., 1990). There are various views which suggest that, for the occurrence of osteopenia in cortical bone, 1 year (Li et al., 1997) or a minimum of 6 months (Yamazaki & Yamaguchi, 1989) should pass after the ovariectomy. Although it is suggested that, due to lack of Havers channels in young rats, old rats have a higher tendency of bone loss as a result of cortical remodeling (Ruth, 1953).

Prostaglandin treatment (Jee et al., 1990) and Ca insufficiency (Ruth) may also affect intracortical remodeling in young rats. Therefore, cytokinin and prostaglandin...
changes, which are suggested to be caused by estrogen withdrawal in ovariectomy (Raisz et al., 2003) may affect cortical remodeling and, may cause early bone loss in the femoral midshaft.

It was reported that, in rats, femur failure loads are mainly associated with total BMD, cross-sectional moments of inertia, cortical bone thickness and cortical bone area (Jiang et al., 2008). In the femoral midshaft, we found a significant correlation between BMD and Lmax. The decrease in Lmax found in the femoral midshaft may be related to the decrease in BMD.

The effect of estrogen depletion on the BMD of the mandible is controversial. Total mandibular BMD was demonstrated to be unchanged (Kuroda et al., 2003; Elovic et al., 1994; Elovic et al., 1995a) notwithstanding the regional decreases indicated (Elovic et al., 1995b), whereas alveolar BMD was reported to reveal a decrease (Yang et al., 2003; Tanaka et al., 2002) even though the detected decrease was shown to be low in a study (Kuroda et al.). The BMD of the molar alveolar region did not change in our study, as the failure load well, which was reported not to be affected by OVX in the previous studies (Elovic et al., 1994; Elovic et al., 1995b).

Mechanical loading during mastication influences bone mass and architecture of the normal and ovariectomized rat mandibles (Mavropoulos et al., 2007, 2005; Elovic et al., 1995a). Ad-libitum fed ovariectomized rats were shown to eat 10% more than SHAM rats (Wronska et al., 1987). In our study, the fact that body weight increased in ovariectomized rats indicates that food intake and masticatory function increased. The reason why Lmax and BMD do not decrease in AR may be that the increased mechanical strength as a result of excessive chewing inhibited the direct effects of estrogen deficiency by controlling bone remodelling and preventing bone loss. In this respect, BMD increase in the ovariectomized rat in our study is notable even this increase is slight and insignificant.

Naproxen treatment prevented the decrease in BMD and maximum load of femoral midshaft caused by OVX in our study. In short-term and long-term studies which analyzed the effects of N and other NSAIDs in bone losses caused by OVX and other reasons, it was found that the loss in both BMD and vertebral compressive strength was partially prevented (Lane et al., 1990; Lane et al., 1994; Jiang et al., 1998; Zeng et al., 1993).

It was suggested that in bone loss related to estrogen deficiency, cytokines and prostaglandin-E2, whose production and sensitivity increased, have a role (Horowits, 1993). The decreasing effect of cyclooxygenase inhibitor naproxen on bone loss and protective effects on the bone may result from inhibition of prostaglandin and cytokine biosynthesis (Martínez et al., 2001). The fact that N was found to decrease osteoclastic activity (Lane et al., 1990) is a concern.

Naproxen was found to significantly inhibit the decrease in the femoral Lmax in ovariectomized rats. The fact that naproxen reduced the loss in the Lmax of femur induced by OVX can be explained by the decreasing BMD loss in this study. However, Lmax is dependent not only on bone mass and density (BMD) but also on bone structure and architecture (Jiang et al., 2008). In this matter, what we only know is that, N does not affect cross-sectional moments of inertia (CSMIs) which was found to be increased in the femoral midshaft of ovariectomized rat (Jiang et al., 1998).

In the present study, no effect of naproxen was found on femur volume and ash density, which are the factors effecting strength. For the determination of the protective effect of N on femur strength, further studies are required which analyze geometric parameters in addition to BMD.

Naproxen did not affect the BMD and Lmax, in the molar alveolar region and femoral midshaft, in SHAM rats. These findings are consistent with the studies which suggest that NSAIDs and N are not effective on normal bone tissue and development (Lane et al., 1990; Solheim et al.; Jee et al., 1988). The reason for this is probably that there is no excessive prostaglandin production, which needs to be blocked in normal bone tissue.

In conclusion, naproxen treatment has an ameliorating effect on the strength characteristics of femoral midshaft afforded by OVX, however, molar alveolar region of the mandible is not affected by either OVX or naproxen.

**RESUMEN:** El objetivo del presente estudio fue investigar los efectos de la ovariectomía y el tratamiento con naproxeno sobre la resistencia de la parte media de la diáfisis femoral y la región alveolar molar en ratas. *Int. J. Morphol.*, 27(3):659-666, 2009.

**TURKOZAN, Y.; ULUSOY, A. T.; BALCIÖGLU, H.A.; BAL, F. & OZEL, S.** Efectos de la ovariectomía y tratamiento con naproxeno sobre la resistencia de la parte media de la diáfisis femoral y la región alveolar molar en ratas. *Int. J. Morphol.*, 27(3):659-666, 2009.

**Introducción:** El objetivo del presente estudio fue investigar los efectos de la ovariectomía y el tratamiento con naproxeno sobre la resistencia de la parte media de la diáfisis femoral y la región alveolar molar en ratas. Fueron utilizadas ratas Sprague-Dawley, la que se dividieron en cinco grupos: referencia, ovariectomizadas simuladas, ovariectomizadas, ovariectomizadas simuladas + tratamiento naproxeno, ovariectomizadas + tratamiento naproxeno. Las mandíbulas y los fémures de las ratas fueron extraídos y se determinó la densidad mineral ósea (DMO) de las muestras extraídas. Los volúmenes y pesos de la ceniza fueron estimados. Las cargas máxi-
mas de las mandíbulas y fémures se determinaron utilizando un ensayo de flexión de tres puntos. La ovariec tomía disminuyó la densidad mineral ósea de la diáfisis media del fémur; sin embargo, el naproxeno impidió esta disminución. Ninguna ovariec tomía sin tratamiento de naproxeno afectó a la DMO en la región alveolar molar. La carga máxima se encontró significativamente disminuida en las diáfisis femoral media, pero por el tratamiento de naproxeno no se produjo esta disminución. La carga máxima de la región alveolar molar no cambió en ninguna condición. El naproxeno previene los cambios de resistencia causados por la ovariec tomía de la diáfisis media del fémur. Sin embargo, ni la ovariec tomía ni el naproxeno tienen un efecto en la región alveolar molar de la mandíbula.

PALABRAS CLAVE: Ovariectomía; Naproxeno; Densidad mineral ósea; Máxima de carga.

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