Bone Mineral Density Changes after Ovariectomy in Rats as an Osteopenic Model: Stepwise Description of Double Dorso-Lateral Approach

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Objective: This study describes a method for inducing osteopenia using bilateral ovariectomy (OVX), which causes significant changes in bone mineral density (BMD) in rats.

Methods: Twenty-five 10-week-old female Sprague Dawley rats were used. Five rats were euthanized after two weeks, and BMD was measured in their femora. The other 20 rats were assigned to one of two groups: a sham group (n = 10), which underwent a sham operation, and an OVX group (n = 10), which underwent bilateral OVX at 12 weeks of age. After six weeks, five rats from each group were euthanized, and BMD was measured in their femora. The same procedures were performed in the remaining rats from each group eight weeks later.

Results: The femur BMD was significantly lower in the six-week OVX group than in the six-week sham group, and in the eight-week OVX group than in the eight-week sham group.

Conclusion: Bilateral OVX is a safe method for creating an osteopenic rat model. The significant decrease in BMD appears six weeks after bilateral OVX.

KEY WORDS: Animal model • Osteoporosis • Rat • Ovariectomy.
cage, in a specific pathogen-free room with a 12-hour light-dark cycle. The rats were allowed free access to tap water and commercially standard rodent food.

**Experimental design**

After two weeks, five 12-week-old rats (control group) were euthanized, their femora were removed, and BMD was measured in the femora. The other 20 rats were assigned to one of two groups. The sham group (n = 10), underwent a sham operation at 12 weeks of age, and the OVX group (n = 10), underwent bilateral OVX, to induce osteopenia, at 12 weeks of age. Six weeks after the OVX or sham operation, five rats from each of the two groups were euthanized, and the femora were removed and BMD was measured. Eight weeks after the OVX or sham operation, the remaining rats in each group were euthanized and BMD of their femora was measured (Fig. 1). All rats were euthanized using CO2 inhalation.

**OVX procedure**

Anesthesia was induced with 5% isoflurane and maintained with 2.5% isoflurane; Oxygen was supplied through a coaxial nose cone during anesthesia. We performed bilateral OVX using a double dorso-lateral approach. Anesthetized rat was laid prone on the operating table and fixed using sticking plaster. The bulged area on the back was shaved bilaterally (Fig. 2A). The ovaries were found on both sides of the abdomen, a little below the kidney; we chose as the skin incision site a position just medial to the most bulging part of the back. This site is less obvious in very young or thin rats, which may lack the bulge. To make the incision, a thumb was placed at the uppermost proximal area of the thigh. The medial portion of the base of the distal phalanx was the incision site (Fig. 2B). A 1.5 cm skin incision was made to expose the dorsolateral abdominal muscles such as the external oblique muscle (Fig. 2C). Entrance to the peritoneal cavity was gained by dissecting the muscle, which revealed the adipose tissue surrounding the ovary (Fig. 2D). The surrounding fat must be gently pulled to avoid detachment of small pieces of ovary (Fig. 2E, F). This shows ovary (thick black arrow) and uterine horn (dotted arrow) surrounded by fat (F). Ligation must undergo at distal uterine horn in order to get rid of total ovary at time (G). Ovary surrounded by fat is removed totally (thick black arrow) (H).

**Fig. 1.** Experimental groups and time schedule. Twenty-five 10-week-old female rats are housed initially. Two weeks later, five rats are sacrificed obtaining femora to determine base-line bone mineral density (BMD). Remained rats are randomly divided into two groups and each group undergoes sham operation and ovariectomy (OVX) respectively. Six and 8 weeks later, five rats from the each two groups are euthanized to get femora that are used in measuring BMD after OVX. *The time of euthanasia.

**Fig. 2.** Procedures of ovariectomy in rat. Anesthetized rat is laid prone on operating table. Thick black arrow : shaving site (A). Skin incision point is located just medial portion of the most bulged area (★) or a thumb used to find the incision point (dotted arrow) (B). External oblique muscle is exposed after skin incision. Thick black arrow : External oblique muscle (C). After the muscle dissection, peritoneal space and adipose tissue surrounding ovary are exposed. Dotted arrow : adipose tissue surrounding ovary (D). The surrounding fat must be gently pulled to avoid detachment of small pieces of ovary (E). This shows ovary (thick black arrow) and uterine horn (dotted arrow) surrounded by fat (F). Ligation must undergo at distal uterine horn in order to get rid of total ovary at a time (G). Ovary surrounded by fat is removed totally (thick black arrow) (H).
Femur BMD measurement

Rats were sacrificed at 12, 18, and 20 weeks, and both femora from each rat were harvested. BMD was measured in the femora using a dual-energy X-ray absorptiometer (DEXA; Lunar PIXImus, Madison, WI, USA) and Lunar PIXImus 2.2.0 software.

Statistical analysis

Statistical analysis was performed using SPSS software (version 12.0, Chicago, IL, USA). The Mann-Whitney test was used to compare values between two groups (sham and OVX), and the Wilcoxon signed-rank test was used to compare mean values of BMD within the same group. Probability values < 0.05 were considered significant.

RESULTS

There was no procedure-related death in this study. Baseline BMD measured in the 12-week-old rats (control group) was 0.184 ± 0.0098 g/mm². The mean femur BMD was significantly lower six weeks after OVX in the OVX rats than in the corresponding sham rats (0.184 ± 0.0098 g/mm² vs. 0.247 ± 0.0482 g/mm², p = 0.000). The mean femur BMD was also significantly lower eight weeks after OVX in the OVX rats than in the corresponding sham rats (0.195 ± 0.0138 g/mm² vs. 0.271 ± 0.0531 g/mm², p = 0.000) (Table 1).

DISCUSSION

Many elderly women experience postmenopausal osteoporosis, and understanding the progression of osteoporosis is helpful for achieving a good outcome after spinal fusion in patients with osteoporosis. Animal models of osteoporosis are crucial for anticipating a treatment’s efficacy and safety as measured by bone quality. OVX is the most frequently used model for studying the events associated with postmenopausal osteopenia. The rat skeleton is more sensitive to the decrease in BMD occurs within six weeks after bilateral dorsal skin incision, we chose the double dorso-lateral skin incisions. This article reports the stepwise description of OVX using the double dorso-lateral skin incisions.

When using an OVX rat model, researchers may be uncertain about the appropriate age and when BMD decreases significantly after OVX. The skeletal response to OVX is more responsive in the growing than in the aged female rat. Because the endocrine system and genital gland in rats mature at the age of three months and the muscles and skeleton are well formed at this time, an OVX model should use rats older than three months. Therefore, we selected 12-week-old rats as the experimental model in this study. Some reports have shown a significant decrease in bone mass four weeks after OVX in the rat and marked decreases in BMD eight or 10 weeks after OVX. Although we observed a significant decrease in BMD six weeks after OVX, the typical osteoporotic profile is seen in bone marrow eight weeks after OVX. Therefore, a better rat model would be to measure BMD and other bone-related variables eight weeks after OVX when evaluating antiresorptive drugs or procedures. We did not evaluate the change in cellularity in the bone marrow after OVX, and bone marrow pathology should be evaluated in future studies.

CONCLUSION

Using bilateral OVX, the osteopenic rat model can be achieved easily, which we described in detail. A significant decrease in femur BMD occurs within six weeks after bilateral OVX in the rat.

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