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Site-Specific Response of Bone Tissue to Ovariectomy in a Rabbit Model

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Abstract

Objective: To explore the changes of bone mass and bone mineral density in different bones of New Zealand female rabbits after bilateral ovariectomy.

Materials and Methods: Twenty female New Zealand rabbits were included and randomly divided into two groups: bilateral ovariectomy or sham operation. Six months after operation, the rabbits were sacrificed and the bone samples of parietal bone, maxilla, mandible, vertebrae, tibia and femur were collected. X-ray, Micro-CT, histology, biomechanical testing and inorganic substances detection were used to evaluate the changes of bone tissue at different sites.

Results: Compared to control, long bones and vertebrae showed significantly decreased bone density, and inorganic substance. BV/TV of the femur decreased by 48.5%, the vertebrae decreased by 45.7%, and the tibia decreased by 32.5%. The inorganic substance in femur decreased by 30.8%, that in tibia decreased by 30.3%, and that invertebrae decreased by 22.0%. Biomechanical results also showed the most decreased mechanical property of long bone and vertebrae, while the parietal bone and jaw bone showed no significant change.

Conclusion: Six months after ovariectomy in a rabbit model, the long bone and vertebrae showed the most loss of bone mass, bone mineral density and mechanical property, while the parietal bone, maxilla and mandible showed no significant change.

Keywords: Osteoporosis; Animal model; Ovariectomy; Bone mineral density; Biomechanics

Introduction

Osteoporosis is one of the most common systemic bone diseases in middle-aged and elderly people, characterized by loss of bone mass and destruction of microstructure of bones, which increased the fragility of bones and the risk of fracture [1]. Low bone mass and microstructure destruction are the main risk factors of osteoporotic fracture [2]. Osteoporosis is more common in middle-aged and elderly people, especially postmenopausal women. Studies have shown that about 40% of osteoporosis patients occurred fractures in hip, waist or wrist, and the occurrence rate is two to three times in women than in men [3-5]. Osteoporotic fracture has posed a great threat to the health of middle-aged and elderly people, and has become one of risk factors that may reduce quality of life, increase disability rate and mortality rate of middle-aged and elderly people.

Ovarian excision to establish an animal model is often used by scholars to simulate the loss of bone mass in postmenopausal osteoporosis patients, including mice, rabbits, pigs, sheep, dogs etc. The mechanism of ovariectomy to establish osteoporosis animal model, is based on the estrogen level drops rapidly after ovariectomy, gradually losing the function of promoting maturation of osteoblasts, and inhibiting bone resorption due to osteoclasts. Result in activating osteoclast, accelerating bone resorption, therefore, balance of osteogenesis-osteoclasts is broken, eventually lead to bone loss, bone microstructure damage.

Preliminary studies have shown that changes are not exactly the same in different bones in animal model of osteoporosis, bone mass loss occurred earlier and worse in long bones and vertebrae than in maxilla and mandible [6,7]. The castrated rat as the most frequently used animal model of osteoporosis, bone loss was similar in the long bones and lumbar spine, among them the tibia and femur were most significant, while bone loss in the maxilla, mandible and parietal bones were slight [8]. Different animals may have different responses to ovariectomy surgery. No studies have compared the bone loss in different sites of ovariectomy rabbit osteoporosis model. In our study, the osteoporosis model was established by removing bilateral ovaries of New Zealand rabbits,

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the samples were taken 6 months after the ovariectomy. And the changes of bone tissue were evaluated by X-ray, Micro-CT, histology, biomechanical testing and mineral analysis.

Materials and Methods

Animals and groups

Twenty male 6 months old about 2.241 ± 0.150 kg white New Zealand rabbits were selected. The animals were kept in an environment complying with the criterions: Temperature 22°C to 24°C, humidity 50% to 60%, day and night per 12 h alternated. The animals were fed for one week on a free diet to adapt to the feeding environment, and then were randomly divided into two groups: The ovariectomy group and the sham operation group, with 10 animals in each group.

Ovariosteresis procedure

The animals underwent bilateral ovariectomy or sham surgery under sterile conditions. The animals were anesthetized by intramuscular injection of Zoletil®50 (Viebac, France) with a dose of 10 $mgkg^{-1}$ to 15 $mgkg^{-1}$ body weight, New Zealand rabbits were lying prone on the operating table, local anesthesia was administered with 2% lidocaine hydrochloride injection in the area of skin incisions. About 2 cm skin incisions were made on the back about two transverse fingers from the midline and 4 cm from the ilium, then subcutaneous fascia was discreetly separated and abdominal muscles were incised about 3 cm to the side of the spine to expose the enterocoelia. After finding the fallopian tube in the yellowish adipose tissue, along the fallopian tube, the morular ovary can be found. The ovaries were completely removed by ligaturing the fallopian tubes and around vessels with 3-0 sterile silk thread, then sutured muscles and skin in layering counterpoint. In the sham operation group, only removed part of the adipose tissue around the ovary. The animals were allowed to eat freely after recovery from anesthesia, and intramuscularly injected cefuroxime sodium for 7 consecutive days to prevent infection.

Sample collection and testing

The animals were sacrificed 6 months after the ovariectomy, and the parietal bone, maxilla, mandible, and tibia, femur and third to fifth lumbar vertebrae were collected and tested by X-ray, Micro-CT, histology, biomechanical testing and mineral analysis.

Histology

All samples of bones were fixed in 4% neutral paraformaldehyde for 72 h. After the fixed solution was removed by PBS, the samples were gradually dehydrated in 40%, 50%, 70%, 80%, 90%, 100% ethanol for 1 h in each solution and embedded with methyl methacrylate. Sections were performed using a tissue slicer (Leica RM2265, Germany) and were stained with Hematoxylin-Eosin (H&E), optical microscope and imaging system (Nikon 80i, Germany) were used for observation.

Micro-CT

The collected bone samples were scanned by a Micro-CT machine (Quantum GX, working voltage 90 kV, working current 88 μA, distinguishability 50 μm), 3D reconstruction and data analysis were performed after scanning. Due to the unclear boundary between cortical bone and cancellous bone of the parietal bone, a square area with a full-layer thickness about 10 mm × 10 mm of parietal bone was selected as the Volume of Interest (VOI). The alveolar bone between the first molar and second molar was selected as the VOI in the maxilla and mandible. The area about 250 μm below the epiphyseal plate in the distal end of femur and proximal end of tibia were selected as VOI in long bones. The cancellous bone in the middle of the vertebral body was selected as VOI. Due to the interferences of teeth in maxilla and mandible, and the limited trabeculae within the parietal bone, the relative Bone Volume (BV/TV ratio) ratio was confirmed to quantitatively analyze in the VOI, and the microstructure of trabeculae was not analyzed in this study [9-11].

Mineral analysis

The samples were dried in the oven at 120°C to constant weight and weighed with a high- precision analytical balance. The samples were placed in a crucible and ashes in a Muffle furnace. The calcination condition was 950°C for 5 h. Then, the ash was weighed by a high-precision analytical balance to calculate the corresponding content (g) of the inorganic substances per 100 g of dry bone.

Biomechanical testing

The samples collected were immersed in normal saline and biomechanical testing were performed on the same day. Biomechanical testing of maxilla, mandible and parietal bone were not carried on because of the interferences of teeth and the limited trabeculae. The samples were placed on the loading platform, and the femur and tibia were tested for three-point bending by gradually applying pressure until the shaft broke, then the maximum bending load (N) and maximum bending strength (MPa) were measured and recorded. A compression test was performed on the vertebral part of the vertebrae, axial pressure was applied vertically to the vertebrae until occurred compression fracture, and the maximum compressive load (N) and maximum compressive strength (MPa) were measured and recorded.

Statistical analysis

All experimental results were showed in the form of mean ± standard deviation, and statistical analysis was performed by SPSS19.0 software (IBM Corp, Armonk, NY, USA). Independent sample t-test was used to contrast the data of experimental group and control group, $p<0.05$ for differences statistically significant.

Results

X-ray

Conventional X-ray can be used to estimate bone mineral density. Six months after the ovariectomy and sham surgery, results showed the bone mineral density of the long bones and vertebrae of the experimental group decreased, and a slight decrease in the parietal bone. While the bone mineral density changes in maxilla and mandible could not be accurately judged due to the interference of the teeth (Figure 1).

Table 1: Micro-CT quantitative analysis of different parts of bone tissue between the control group and the experimental group 6 months after castration operation.

BV/TV	Control group	Ovariectomy group
Parietal bone	16.0 ± 4.71	14.1 ± 3.59
Maxilla	43.5 ± 6.26	40.7 ± 4.73
Mandible	42.6 ± 8.36	42.0 ± 6.63
Vertebra	32.2 ± 4.71	17.5 ± 3.62*
Femur	23.3 ± 3.73	12.0 ± 4.5*
Tibia	24.0 ± 4.52	16.2 ± 3.3*

Data were expressed as mean ± standard deviation, and independent sample T test was used for inter-group comparison. An asterisk indicates significance at $p<0.05$. BV/TV ratio indicates relative bone volume

Table 2: Results of inorganic substance analysis of different parts of bone tissue between the control group and the experimental group 6 months after castration operation.

Inorganic substance (g/100 g of dry bone)	Control group	Ovariectomy group
Parietal bone	53.8 ± 7.36	53.0 ± 8.47
Maxilla	50.8 ± 11.63	47.8 ± 5.85
Mandible	51.0 ± 11.84	47.5 ± 8.57
Vertebra	54.4 ± 7.47	42.6 ± 6.78*
Femur	54.6 ± 4.35	37.8 ± 8.45*
Tibia	56.7 ± 3.76	39.5 ± 6.45*

Data were expressed as mean ± standard deviation, and independent sample T test was used for inter-group comparison. An asterisk indicates significance at p<0.05

Table 3: Biomechanical test results of different parts of bone tissue between the control group and the experimental group 6 months after castration operation.

	Control group	Ovariectomy group
Maximum compression/ bending strength (Mpa)		
Vertebra	640.6 ± 111.56	301.5 ± 79.98*
Femur	380.5 ± 36.86	251.9 ± 28.97*
Tibia	413.7 ± 35.23	286.8 ± 27.67*
Maximum compression/ bending load (N)		
Vertebra	26.7 ± 5.34	12.6 ± 2.97*
Femur	44.6 ± 5.89	31.3 ± 2.42*
Tibia	88.1 ± 9.78	62.8 ± 7.45*

Data were expressed as mean ± standard deviation, and independent sample T test was used for inter-group comparison. An asterisk indicates significance at p<0.05

Histology

After 6 months of ovariectomy and sham surgery, a great change had taken place in trabeculae morphology of long bones and vertebrae in the experimental group, in the way of thinning of trabeculae, reduction of amount, partial disconnection, and decrease in bone mass density. The amount and structure of the trabeculae in parietal bone, maxilla and mandible were not obvious (Figure 2).

Micro-CT

Six months after ovariectomy and sham surgery, in the experimental group, the trabeculae of long bone and vertebrae was thinner and less continuous. And the amount decreased and the morphology damaged was also observed in the trabeculae of parietal bone, maxilla and mandible (Figure 3). Quantitative analysis results showed that the degree of BV/TV ratio decreased were significantly different in bones, femur decreased by 48.5%, vertebrae decreased by 45.7%, tibia decreased by 32.5%, while parietal bone, maxilla and mandible hardly changed, the differences were not statistically significant (Table 1).

Mineral analysis

Six months after ovariectomy and sham surgery, compared with the control group, the content of inorganic substances decreased significantly in the experimental group, the femur decreased by 30.8%, tibia decreased by 30.3% and vertebrae decreased by 21.7%, while parietal bone, maxilla and mandible hardly changed, the differences were not statistically significant (Table 2).

Biomechanical testing

Six months after ovariectomy and sham surgery, in the experimental group, the maximum compressive load of vertebrae decreased by 52.9%, the maximum compressive strength decreased by 52.8%, the maximum bending load of femur decreased by 33.8%, the maximum bending strength decreased by 29.8%, the maximum bending load of tibia decreased by 30.7%, and the maximum bending strength decreased by 28.7% (Table 3).

Discussion

The castrated rabbit is a commonly used to establish postmenopausal animal model of osteoporosis, and the animal model of osteoporosis usually stabilize 3 to 6 months after ovariosteresis [12-14]. In order to fully demonstrate the effect of ovariectomy surgery on bone tissues, the samples were collected 6 months after ovariectomy surgery in this study. Preliminary studies have shown that effects of ovariectomy surgery on bone tissue in different parts of emasculated rats are significantly different [8], however, it remains unclear whether there are statistical differences in the effects of ovariectomy on bone tissue in different parts of rabbits, therefore, and this study focuses on exploring this question. In order to comprehensively evaluate the influence of ovariectomy surgery on different bone tissue of rabbit model of osteoporosis, we selected long bones (femur, tibia), vertebrae, parietal bone, maxilla and mandible as the research objects, nearly covering the bones commonly used in other different experiments.

The changes of bone tissue include bone mass, bone mineral density, bone fiber structure, bone mineral content, bone mechanical properties and so on. Therefore, X-ray, Micro-CT and other imaging tests, histological observation, inorganic content of dry bone calcined and biomechanical testing were used to detect different bone tissues. Different detection methods consistently showed that ovariectomy surgery had the greatest effect on the long bones and vertebrae, and had little impact on the parietal bone, maxilla and mandible. Micro-CT quantitative analyses showed the bone mass of distal femur in the experimental group has the most reduction, BV/TV ratio decreased by 48.5%, vertebrae followed with a 45.7% reduction in BV/TV ratio. The results of dry bone calcination also

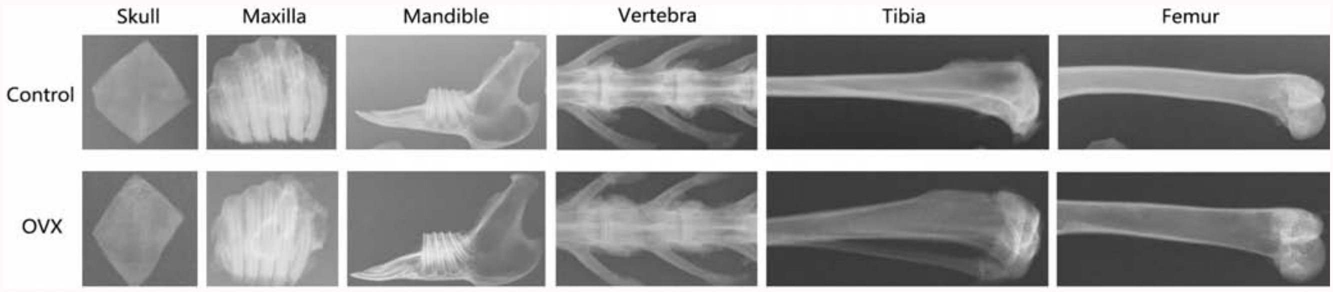

Figure 1: X-ray results of bone tissue from different sites 6 months after sham operation or ovariectomy.

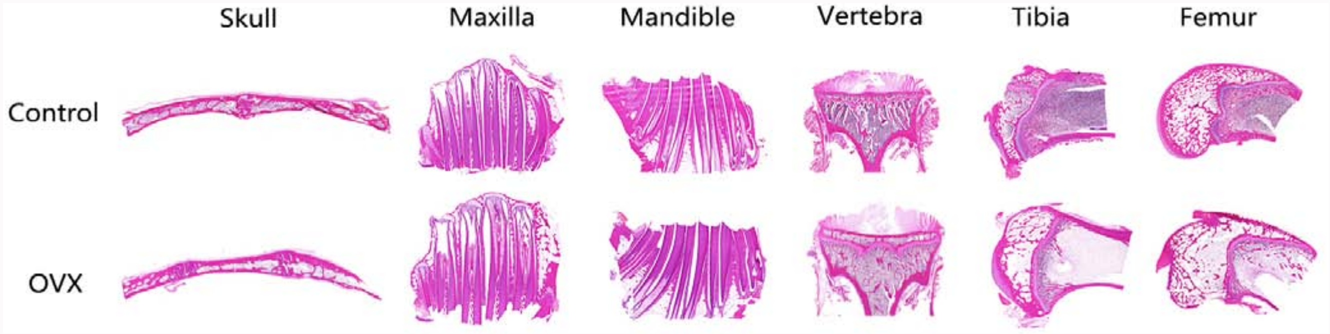

Figure 2: Histological results of bone tissue from different sites 6 months after sham operation or ovariectomy.

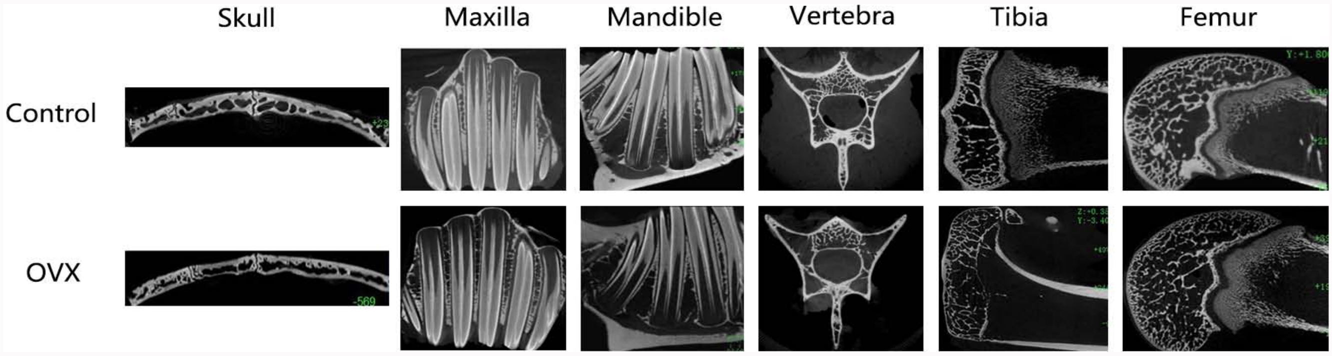

Figure 3: Micro-CT slices of bone tissue from different sites 6 months after sham operation or ovariectomy.

showed that the inorganic content of femur decreased the most, reaching 30.8%, tibia decreased by 30.3% and vertebrae decreased by 21.7%. However, the biomechanical testing results showed that the greatest decrease in mechanical properties was the vertebrae, whose maximum compressive load decreased by 52.9% and maximum compressive strength decreased by 52.8%. It seems revealed the decrease of biomechanical properties of bones were not absolutely determined by bone mass and mineral content. The changes caused by microstructure and the ratio of cortical bone to cancellous bone may also play a part. Vertebrae is rich in cancellous bone is the biggest difference with femur, without strong cortical bone like femur, this partly explains why osteoporotic fractures are more common in the lumbar spine [15].

An interesting finding, the tolerance of maxilla and mandible to ovariectomy surgery is higher than that of long bones and vertebrae, which is consistent with the results of clinical studies. One-third of postmenopausal women experience at least once spontaneous osteoporotic fracture or caused by low-impact event in their lifetime. However, osteoporotic fracture of jaws are rare, this may be related to the different biological characteristics of bone precursor cells in different bone tissues [16]. It is reasonable to apply metaphysis of the femur or tibia in the field of osteoporosis related experiments, for ovariectomy has a more significant effect on rabbit long bones [17,18].

The final biomechanical properties of bone tissue may be closely related to mass, density and microstructure. In the quantitative analysis of Micro-CT examination, it was easy to analyze the trabecular microstructure of the epiphysis and vertebrae. But it was hardly possible to analyze trabecular microstructure accurately and effectively due to the interferences of teeth in maxilla and mandible, and the limited trabeculae within the parietal bone. For similar reasons, biomechanical analyses of the parietal bone, maxilla and mandible were not completed; these are the things that we regret about the design of this experiment. In addition, there were no significant changes in the parietal bone, maxilla and mandible 6 months after the ovariectomy operation, but if the observation period was extended, it might be possible to see significant changes.

Conclusion

Six months after the female New Zealand rabbit's ovariectomy, the bone mass, bone mineral density, mineral content and mechanical properties of long bone metaphysis and vertebrae decreased significantly, but the changes of parietal bone, maxilla and mandible were not obvious. The results provide a reference for the selection of observation sites in relevant experiments using castrated rabbits as research objects.

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