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Research Article

Suitable Ovariectomy Age for Screening the Functional Agents by Femoral Bone Strength in Osteoporosis Model Rats

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Abstract

Osteoporosis is a major contributor to the high frequency of bone fracture in elderly women. The ovariectomized (OVX) rat is one of the excellent pre-clinical animal model of osteoporosis. Following ovariectomy, rapid loss of cancellous bone mass and strength occurs. Maximum breaking force of the femoral diaphysis is a major parameter to determine the severity of the osteoporosis directly. Although, the suitable age at ovariectomy remains obscure to evaluate the maximum breaking force of the femoral diaphysis. Accordingly, the suitable rat age at ovariectomy to evaluate the maximum breaking force of the femoral diaphysis for screening of therapeutic or functional agents was determined. Female Sprague-Dawley rats (6, 13 or 30 week-old) were used. Rats of each age were divided into two groups; underwent bilateral OVX and underwent bilateral laparotomy (sham). All rats were fed an AIN93G-based normal diet for further 10 weeks. Reduction of maximum bone strength in femur and increase of body weight gain were observed only in 6 week-old OVX rats after 10-week acclimatization, whereas the difference was obscure in 13 and 30 week-old OVX rats. Therefore, ovariectomy at 6 week-old was the suitable age for osteoporosis model to screen the effects of functional agents in rats.

Keywords: Bone strength; Femur; Osteoporosis; Ovariectomy; Rat; Serum calcium concentration

1. Introduction

Osteoporosis is a disease distinguished by a decrease in bone mass (osteopenia) and a degradation in bone micro-architecture which leads to an enhanced fragility of the skeleton, and therefore to a

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greater risk of fracture (Kanis, 1994; Jee and Yao, 2001) Osteoporosis is a major contributor to the high frequency of bone fracture in elderly women. The multiple factors implicated in osteoporosis, its obscure pathogenesis, the dramatic decline in quality of life, high incidence of the disorder, financial cost, and high mortality, therefore require for further experimentation in animal models imperative (Lelovas et al., 2008).

The ovariectomized (OVX) rat is one of the excellent pre-clinical animal model that precisely follows the clinical feature of the estrogen depleted human skeleton and the response of therapeutic agents (Jee and Yao, 2001; Torricelli et al., 2004). Following ovariectomy, rapid loss of cancellous bone mass and strength occurs, therefore the many investigators were analyzed the cancellous bone volume to evaluate effectiveness of the therapeutic or functional agents in OVX rats (Danielsen et al., 1993). Maximum breaking force of the femoral diaphysis is also one of the major parameters to determine the severity of the osteoporosis directly (Mitamura and Hara, 2005; Li et al., 2009). Although, the suitable age at ovariectomy remains obscure to evaluate the maximum breaking force of the femoral diaphysis, whereas the analysis of cancellous bone volume was already recommended the suitable age at ovariectomy in the review of osteoporosis model study for rats (Jee and Yao, 2001). Accordingly, we determined the suitable rat age at ovariectomy to evaluate the maximum breaking force of the femoral diaphysis for screening of therapeutic or functional agents in this study.

2. Materials and Methods

2.1 Animals and diets

The experimental design was same as described previously except for the ages of animals (Matsukawa et al., 2011). Female Sprague-Dawley rats (6, 13 or 30 week-old; Japan Clea, Tokyo, Japan) were housed in individual stainless-steel cages with wire-mesh bottoms. The cages were placed in a room with controlled temperature (23-25°C), relative humidity (40-60%) and lighting (lights on 08:00-20:00 hours). The rats had a semi-purified diet based on the AIN93G formulation for an acclimation period of 5 days. The experimental animals were handled in accordance with the guidelines for animal studies of the Kyoto Institute of Nutrition and Pathology (Kyoto, Japan).

2.2 *Experimental design*

Rats of each age were divided into two groups, underwent bilateral ovariectomy (OVX) and underwent bilateral laparotomy (sham). The number of rats in each group was shown in Table 1. All rats were fed an AIN93G-based normal diet for further 10 weeks. Food intake of OVX rat in each age was adjusted to the average intake in the sham group of corresponding age in each day. All rats were given free access to deionized water throughout the experiment. The body weight was measured every week. On the last day, the rats were anesthetized (overdose of sodium pentobarbital; Somnopentyl, Kyoritsu, Tokyo, Japan), and were sacrificed after collection of aortic blood. Blood was centrifuged (1,300 ×*g* for 10 min) to obtain the serum. Left femur was then removed, carefully cleaned of adherent tissue, and was used to measure the bone strength. The uterus was removed and weighed to confirm the success of the ovariectomy in each rat. The bone strength was measure by a three-point bending test (Shiga et al., 2002) with a rheometer (RE-3305 Rheoner; Yamaden, Tokyo, Japan) under the following conditions: 1.0 cm of sample space; 30 mm/min of pranger speed; 20 kg of load range. Calcium and phosphorus concentrations in the serum were analyzed by the Japan Clinical Laboratories (Kyoto, Japan) with standard analytical methods.

2.3 Statistical analyses

A complete randomized design 2-way ANOVA was used to analyze the differences in each parameter between the age and OVX. Scheffe's *F* test was used for multiple comparisons when needed for analyses of the "age" factor. The differences among groups were considered significant at *P*< 0.05. All data were analyzed by Statcel (Yanai 1998).

Table 1 Final body weight, body weight gain, and uterine weight of sham and ovariectomized rats among the different ages

Ovariectomized age	OVX	n	Body weight at dissection (g)	Body weight gain (g/10 wk)	Uterine weight (g/ 100 g BW)
6 week-old	−	11	269.4±8.0	139.2±6.8	0.31±0.02
	+	7	298.4±3.8	171.4±3.6*	0.11±0.01
13 week-old	−	10	325.4±5.1	79.9±4.6	0.32±0.03
	+	9	323.7±2.9	82.7±3.8	0.06±0.01
30 week-old	−	6	293.8±17.4	42.7±10.8	0.30±0.04
	+	6	300.2±8.2	56.0±2.8	0.05±0.01
2-way ANOVA p value	Age		<0.001	<0.001	0.91
	OVX		0.004	0.11	<0.001
	Interaction		0.98	0.04	0.97

Values represent means ± SEM. The effect of the two-way interaction (age × OVX) on the body weight gain was significant. Therefore, statistical analysis was applied separately to the results of each age. Student's *t*-test was used to analyze the differences among means for each age. An asterisk represents the significant difference between sham and OVX rats at 6 week-old, *P* < 0.05.

3. Results

3.1 Body weight and body weight gain (Table 1)

Body weight at the dissection day was significantly higher in OVX rats than those in sham-operated rats, while there were no differences in food intake between sham and OVX rats in all ages (Data not shown). Body weight gain was also higher in OVX rats than those in sham rats in 6 week-old group. The uterine weights of OVX rats were significantly lower than those of the sham rats in all ages.

3.2 Maximum breaking force of the femur (Fig. 1)

Relative values (N/100 g BW) in the maximum breaking force of the femur were lower in OVX rats than in sham rats in 6 week-old group, whereas the difference was obscure in the other age groups.

3.3 Calcium and phosphorus concentration in serum (Table 2)

Calcium concentration in serum was affected by OVX; it was lower in OVX group than in sham group in all ages. Phosphorus concentration was not affected by OVX with a significant interaction between age and OVX. The concentration was higher in OVX rats than in sham rats in 13 week-old group.

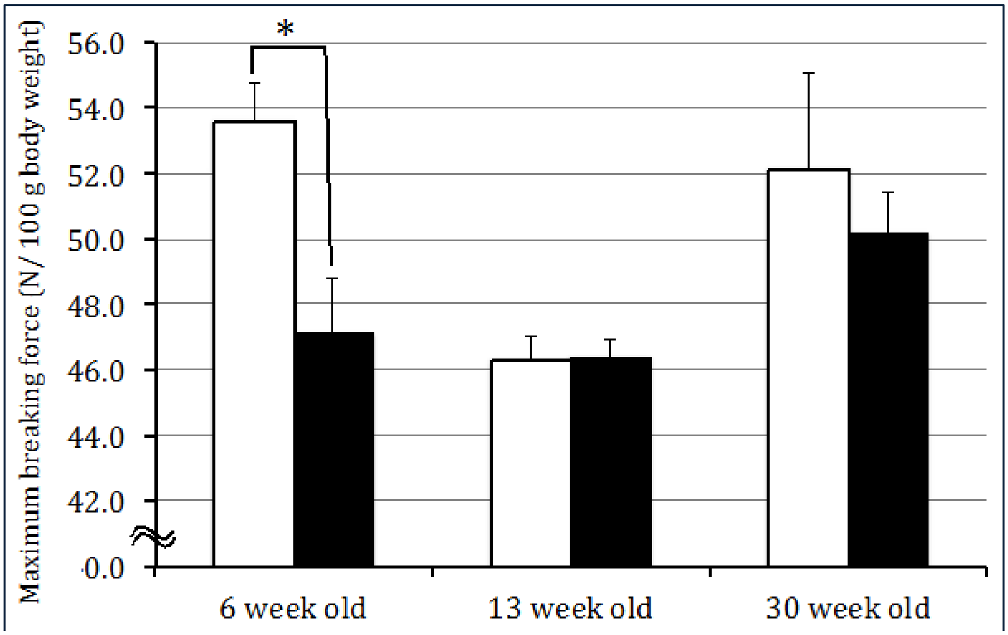

Fig 1. Maximum breaking force of the femur in sham and ovariectomized (OVX) rats among the different ages. Open and closed bars represent the values for sham and OVX rats, respectively. Values are the means ± standard error; n = 11 (6 week-old OVX), 7 (6 week-old Sham), 10 (13 week-old OVX), 9 (13 week-old Sham), 6 (30 week-old OVX), 6 (30 week-old Sham). The effect of the two-way interaction (age × OVX) on the maximum breaking force was significant. Therefore, statistical analysis was applied separately to the results of each age. Depending on the results of the *F*-test, Student's *t*-test or Welch's *t*-test was used to analyze the differences among means for each age. An asterisk represents the significant difference between sham and OVX rats, $P < 0.05$.

Table 2 Serum calcium and phosphorus concentrations of sham and ovariectomized rats among the different ages

Ovariectomized age	OVX	n	Calcium (mg/dL)	Phosphorus (mg/dL)
6 week-old	–	11	10.7±0.1	5.6±0.2
	+	7	9.8±0.1	5.3±0.2
13 week-old	–	10	10.9±0.2	4.9±0.2
	+	9	9.9±0.1	5.9±0.3*
30 week-old	–	6	11.0±0.1	4.7±0.2
	+	6	10.2±0.1	4.7±0.3
2-way ANOVA p value	Age		0.04	0.005
	OVX		<0.001	0.25
	Interaction		0.36	0.03

Values represent means ± SEM. The effect of the two-way interaction (age × OVX) on the phosphorus concentration was significant. Therefore, statistical analysis was applied separately to the results of each age. Depending on the results of the *F*-test, Student's *t*-test was used to analyze the differences among means for each age. An asterisk represents the significant difference between sham and OVX rats at 13 week-old, $P < 0.05$.

4. Discussion

Jee and Yao (2001) were recommended to use 9 month-old OVX rats with evaluation of the cancellous bone volume. Since female rats of this age reached peak bone mass and can be manipulated to simulate clinical findings of post-menopausal osteoporosis. However, commercial supplier of rats can provide approximately 10-weeks or younger age rats at least in Japan, an investigator therefore must keep rats at 9 month-old to perform the Jee and Yao recommendation (2001). According to the economical and commercial viewpoints, the use of young animals to evaluate effectiveness of anti-osteoporosis agents is reasonable because of the low cost and getting speedy results. Therefore we used relatively young animals such as 6 week-old (age generally used in the screening test of the functional agents (Tamura et al., 2006; Yamamoto and Oue, 2006; Kanazawa et al., 2008), 13 week-old [many investigators (Sehmisch et al., 2009; Chiang et al., 2011; Liang et al., 2011) were used for OVX at this age], and 30 week-old (very old but younger than 9 month-old rat) to determine the suitable age at ovariectomy in this study. According to our results, calcium concentration in serum was reduced by ovariectomy in all ages, whereas maximum bone strength was impaired only in 6 week-old OVX rats by this treatment. Furthermore, our resent study has suggested that the up-regulation of the receptor activator of nuclear factor kappa-B ligand (RANKL) mRNA expression was shown in the femur as a key factor for osteoclast differentiation and activation (Suda et al., 1999; Boyle et al., 2003), and also shown the accumulation of the osteoclast by the tartrate-resistant acid phosphatase (TRAP) staining analysis of 6 week-old OVX rats. These studies were performed as the same experimental procedure in this study (Matsukawa et al, Unpublished data). The thickness of compact bone in middle position of the femur was also significantly reduced in 6 week-old OVX rats. Accordingly, the reduction of maximum bone strength in 6 week-old OVX rats observed in the present study may be caused by the osteoclast accumulation and atrophy of compact bone thickness in the femur.

In conclusion, the analysis of femoral maximum breaking force in rats conducted ovariectomy at 6 week-old age is one of the most suitable study design to screen the effects of therapeutic or preventive agents against osteoporosis.

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