Original Article

Effects of soybean isoflavone dosage and exercise on the serum markers of bone metabolism in ovariectomized rats

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This study was designed to determine whether combined treatments with soybean isoflavone dosage and moderate exercise would exhibit synergistically effects on bone metabolism following the onset of menopause. Fifty 12 wk-old female Wistar rats were assigned to five groups: 1) Sham operated (Sham), 2) ovariectomized (OVX), 3) OVX received soybean isoflavone (OVX-IF), 4) OVX exercised (OVX-EXE) and 5) OVX treated with both soybean isoflavone and exercise (OVX-IF-EXE). All rats were fed a normal diet ad libitum. Daily soybean isoflavone dosage was 50 mg/kg body weight. The vehicle was given in Sham, OVX and OVX-EXE groups. The drugs were all oral administered using a stomach tube. Exercising rats were trained on an uphill treadmill at 20 m/min for 1h/day, 5days/week. The experimental duration consisted of the adaptation periods of 2 weeks and treatment periods of 8 weeks. The results showed that the uterus relative weights in OVX-EXE, OVX-IF and OVX-IF-EXE groups were all lower than those in Sham, they were higher than those in the OVX group. Serum alkaline phosphates (AKP) activitie of OVX was significantly increased as compared to that of Sham (p<0.01). OVX-IF and OVX-IF-EXE respectively decreased the Serum alkaline phosphates activities, as compared to that of OVX (p<0.01). The tartrate-resistant acid phosphatase (TRAP) value of OVX was significantly increased as compared to that of Sham (p<0.05). OVX-IF decreased the TRAP as compared to that of OVX (p<0.05). These results suggest soybean isoflavone and resistance exercise both can restrain ovx-induced bone loss. But their mechanisms may be different.

Key Words: ovariectomized rats, soy isoflavone, exercise, bone mineral density

Introduction

Osteoporosis is a major health care problem in the elderly, which is characterized by low bone mass, leading to an increase in risk of fracture. The postmenopausal bone mass is prevented by estrogen administration, but it is often accompanied with side effects such as breast cancer.[1]

Recent studies have shown that nonsteroidal estrogen-like plant compounds called phytoestrogens are effective in preventing osteoporosis in animal models.[2] The major phytoestrogens consumed by humans exist in soybean and are classified as soybean isoflavones. The soybean isoflavones, prevented bone loss caused by estrogen deficiency without substantial effects on the uterus in ovariectomized (OVX) mice.[2]

Physical exercise that loads mechanical stress to the bone is effective also in maintaining bone mass in postmenopausal women.[3] Furthermore, it has been suggested that combined intervention of exercise and estrogen treatment could prevent bone loss both in postmenopausal women and in animal models with osteoporosis. Thus, we presumed that the combined intervention of running exercise and isoflavones administration might be effective in preventing bone loss caused by estrogen deficiency without substantial effects on reproductive organs.

In this study, we examined the cooperative effects of moderate intensity exercise and a submaximal dose of isoflavones on bone metabolism in OVX rats. Our data indicated that combined intervention exhibited cooperative effects on the prevention of bone loss in OVX rats.

Materials and methods

Animal and intervention

Fifty female Wistar rats aged 12 weeks, weighing 233g±16g, were purchased from Tianjin Laboratory Animal Center (Tianjin, China). The rats were housed individually in a room under a 12/12 h light/dark cycle at 22°C and allowed free access to water and diet.

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The animals were either sham or ovariectomy operated within two weeks after arrival, and rando and randomly divided into five groups on the basis of body weight: Sham (sham, n = 10), OVX-control (OVX, n =10), exercise training (OVX-EXE, n = 10), isoflavones administration (OVX-IF, n = 10) and combined isoflavones and exercise (OVX-EXE-IF, n = 10). Soybean isoflavone (Harbin high-tech Corp., Heilongjiang, China) dosage was 50 mg/kg body weight. The vehicle was given in Sham, OVX and OVX-EXE groups. The drugs were all oral administered using a stomach tube 4 days after surgery. The Ex regimen consisted of daily running on a treadmill (Tianjin Institute of Physical Education. Tianjin, China) for 60 minutes/day at 20m/minute up a 5° slope. Eight weeks after the start of intervention, the rats were killed and the weight of uterus was measured. Blood (20ml) was drown from the abdominal aorta for the measurement of serum alkaline phosphatase (AKP), tartrate-resistant acid phosphatase (TRAP) and bone Gla protein (BGP). AKP, TRAP and BGP kits were purchased from the Jiancheng biology Co. (Nankin, China) and the PLA general hospital (Pekin, China), respectively.

Statistical analyses: Data were presented as means ± SEM. The significance of the differences was determined by two-way ANOVA. Differences were considered significant at the level of $p<0.05$, $p<0.01$ and $p<0.001$.

Results

Change of body weight and uterine weight

The five groups of rats started with similar initial mean body weight. The rats in all groups gained weight during a 8-week experimental period. The body weight was significantly higher in the OVX group than those in sham group ($p<0.01$). The uterine weight decreased strikingly in OVX rats ($p<0.001$), indicating that the rats were estrogen deficient. As reported previously, E_2 restored the decreased uterine weight in OVX mice to the same level as that in the sham mice. The uterine relative weight of IF and EXE-IF groups was significantly increased, compared with that of OVX group ($p<0.01$). The combination of isoflavone administration and running exercise did not affect uterine weight either. (Table 1)

Table 1. Effects of soybean isoflavone and exercise on the body weight and uterine of ovariectomized rats

Group	number	rate of the change of body weight(%)	uterine relative weight(%)
Sham	10	18.3±13.1	0.169±0.041
OVX	10	36.4±8.7$^{\#\#}$	0.028±0.003$^{\#\#\#}$
OVX-EXE	10	25.1±8.1*	0.037±0.006*
OVX-IF	10	32.7±6.1	0.045±0.011**
OVX-IF-EXE	10	24.5±9.9*	0.043±0.009**

Note: Compare with Sham, $^{\#}$: $p<0.05$, $^{\#\#}$: $p<0.01$, $^{\#\#\#}$: $p<0.001$; Compare with OVX, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

Serum analysis

The AKP and TRAP activities for the OVX group were higher than that for the Sham group significantly ($p<0.01$, $p<0.05$). The AKP activities for the IF and EXE-IF groups were lower that for the OVX group ($p<0.01$). Compared with OVX, the TRAP activities for the IF, EXE and EXE-IF groups were all decreased, but that for IF was remarkable ($p<0.05$). The difference of the BGP among all groups was not remarkable. There are no significant serum marker changes in EXE group, as compared to that of OVX. (Table 2)

Discussion

This study shows that the combined intervention of moderate exercise and the isoflavone administration exhibit cooperative effects on prevention of bone loss in OVX rats. In this study, we used ovariectomized rats with the initial age of 12 weeks as a postmonopausal model. Kalu DN[4] designated that rats aged 3 months could successfully be used as a mature rat model, because these rats were not only reproductively mature but capable of responding appropriately to sex hormone deficiency and its sequela following ovariectomy. Moreover, the characteristics of the bone loss were mostly similar to those of the aged rat model. The weight of the ovariectomized animals increased rapidly, due to economy resisted the ovariectomized influencing on skeleton. It is a kind of self-protection, may restrain bone loss in OVX rats partially, but could not prevent it's development.[4] Our study shows the weight of OVX rats was increased remarkably, that illuminated the osteoporosis model was succeed. It was accordant with many reports.[5,6]

Table 2. Effects of soybean isoflavone and exercise on the biomarkers of bone metabolism of ovariectomized rats

group	number	BGP（ng/ml）	AKP(U/L)	TRAP(U/L)
Sham	10	9.84±1.80	16.4±2.94	4.39±0.88
OVX	10	9.08±1.49	23.2±3.57$^{\#\#}$	6.71±0.92$^{\#}$
OVX-EXE	10	7.88±1.27	24.7±4.09	5.14±1.13
OVX-IF	10	10.5±2.14	17.1±3.63**	4.44±0.90*
OVX-IF-EXE	10	8.10±1.06	17.9±4.50**	5.03±1.61

Note: Compare with Sham, $^{\#}$: $P<0.05$, $^{\#\#}$:$P<0.01$, $^{\#\#\#}$:$P<0.001$; Compare with OVX, *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$. AKP: serum alkaline phosphates; BGP: bone Gla protein; TRAP: tartrate-resistant acid phosphatase

The effects of running exercise on the body weight of OVX rats was described differently. Nordsletten *et al.*[5] has reported that exercise with 27m/minute intensity might restrained the weight of OVX rats's increase. Peng *et al.*[7] has reported that exercise with 18 m/minute intensity could reduce the gain weight of OVX rats. Zhang *et al.*[8] has reported that exercise with 20 m/minute intensity had not restrainable effect on the weight of OVX rats's increase. And compare with quiet group, moderate intensity rats weight' increase was higher than OVX-C group.

The effects of isoflavone on the body weight of OVX rats was reported consistently. Fanti *et al.*[9] has reported that the body weight of genistein group was increased higher than the Sham and lower than Cont group. Shi *et al.*[10] has reported that the body weight of IF group was higher than Sham 42 days after the ovariectomy.

Our study shows that 50 mg/kg soybean isoflavone dosage and moderate exercise all would reduce the gain weight of OVX rats, the moderate exercise would be more remarkable. ($p<0.05$)

Ishimi *et al.*[11] has reported that genistein prevents bone loss caused by estrogen defieieney without exhibiting estrogenic action in the uterus. But phytoestrogens, including soybean isoflavones, have received considerable attention due to their possible effects as endocrine disrupters. According to our results, isoflavone extract from soybean hypocotyls was able to prevent OVX-induced bone loss without exhibiting estrogenic action in the uterus of OVX-IF rats.

Bone remodeling activity is in contact with bone metabolism parameters. OVX-induced osteoporosis belongs to high conversion type, bone formation and bone resorption are all increased, but bone resorption is more remarkable. AKP and BGP are markers of osteogenesis, TRAP is markers of bone resorption. Our study shows that levels of BGP were not significantly among all the groups ($p>0.05$). And the levels of serum BGP of postmenopausal women were reported inconsistently.[4,12,13] In our study, the AKP and TRAP activities for the OVX group were higher than that for the Sham group significantly ($p<0.01$，$p<0.05$). That shows bone resorption in OVX rats was increased, it appeared compensative bone formation increase accompanying bone resorption's excessive.

Compared with OVX, the AKP activities of the IF and EXE-IF groups were lower than that of the OVX group ($p<0.01$) after 8 weeks, but the difference in EXE group was not remarkable. It shows the isoflavone have mostly effect on bone formation compared with exercise. So the mechanism of the isoflavone and exercise preventing bone loss in OVX rats may be different. Isoflavone prevented osteoporosis likely by restraining bone metabolize high turnover state.

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