Effects of resveratrol on bone metabolism and bone turnover related indexes in ovariectomized osteoporosis rats

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Abstract: This study aimed to investigate the effects of resveratrol (RES) on bone metabolism and bone turnover related indexes in ovariectomized osteoporosis rats. 48 clean grade adult healthy unmated female SD rats were randomly divided into 6 groups, including normal control group (NCG), osteoporosis model group (OP MG), estrogen treatment group (17β-E2 group), RES low dose group (RES-L), RES medium-dose group (RES-M) and RES high dose group (RES-H). The rats in NCG and OP MG were given distilled water once a day and the rats in the other two groups were given 17β-E2 and resveratrol respectively. The levels of serum calcium (S-Ca), serum phosphorus (S-P), urinary calcium (U-Ca/Cr) and urinary phosphorus (U-P/Cr) were measured with an automatic biochemistry analyzer. The levels of serum osteocalcin (BGP), alkaline phosphatase (ALP), tartrate-resistant acid phosphatase (TRAP), type I amino front-end peptide (PINP), type I collagen strong carboxyl peptide (CTX-I), urine deoxypyridinoline (DPD) and serum estrogen were detected by enzyme-linked immunosorbent assay (ELISA). In the OP group, the serum estrogen levels, S-Ca and S-P decreased significantly and the expression of U-Ca/Cr and U-P/Cr increased significantly (P<0.05). Compared with the OP group, the expression of S-Ca and S-P increased significantly and the expression of U-Ca/Cr and U-P/Cr decreased significantly (P<0.05) after treatment. The levels of TRAP, BGP, DPD and CTX-I in the OP group increased significantly (P<0.05). Compared with the OP group, the levels of TRAP decreased significantly (P<0.05). The levels of PINP and ALP in OP MG increased significantly (P<0.05). IP and ALP increased in the middle and lower levels (P<0.05). The bone mineral density of the OP group decreased significantly (P<0.05). Resveratrol can affect the changes in bone turnover in ovariectomized rats, promote bone formation in low estrogen state and inhibit bone resorption. Resveratrol may have a protective effect on the bone of ovariectomized rats.

Key words: Resveratrol; Ovariectomized osteoporosis; Bone metabolism; Bone turnover; Animal model.

Introduction

Osteoporosis is a skeletal disorder that most often occurs in old age and is characterized by a decrease in bone strength and puts a person at risk for fractures. It is common in menopause because women have very low estrogen. Deficiency of minerals such as calcium, protein and vitamin D, and smoking (such as cigarettes) can also cause osteoporosis. In the absence of minerals in the diet, the human body will ingest such minerals from bone tissue. Over time, the bones will become deficient in minerals, resulting in osteoporosis. Moreover, with age, the destruction of bone tissue becomes more than its structure, and as time goes by, this complication will occur in old age. Some diseases, such as hyperthyroidism, hypothyroidism, rheumatoid arthritis, Cushing's disease and diabetes, and the use of certain drugs (such as heparin, phenytoin and other corticosteroids and levethyroxine) can cause osteoporosis. Other factors, such as a sedentary lifestyle or underweight, are more than 10% more likely than young or overweight genetic factors and 19% to cause osteoporosis caused by alcoholism. Excessive caffeine intake is considered a risk factor, but recent studies have shown that there is little connection between osteoporosis and caffeine intake (1-5). Osteoporosis is a systemic bone disease that is prone to fracture due to the decrease of bone density and bone quality, destruction of bone microstructure and increase of bone fragility caused by various reasons (1). Osteoporosis is divided into secondary and primary types. Primary osteoporosis can be divided into postmenopausal osteoporosis (type I), senile osteoporosis (type II) and idiopathic osteoporosis (including adolescents). Postmenopausal osteoporosis generally occurs within 5~10 years after menopause in women. Senile osteoporosis generally refers to osteoporosis after 70 years old in the elderly. Idiopathic osteoporosis mainly occurs in adolescents (2).

RES widely exists in grapes, peanuts, polygonum cuspidate and other plants. It is a natural polyphenolic compound with a similar structure to estrogen diethylstilbestrol. Its chemical name is 3,5,4'-trihydroxystilbene. Vitro experiments showed that resveratrol can competitively bind with estrogen to become estrogen receptor and it has estrogen/antiestrogen effects (3). Therefore, resveratrol is regarded as a plant hormone. The study...
on cardiovascular protection and anticancer effects of resveratrol have become one of the research hotspots in maternal, child and elderly nutrition in recent years (4-6). However, the current data on the effects of resveratrol on bone turnover indexes of climacteric women are still very limited.

In this study, ovariectomy rats were used as animal models to keep the rats in a state of low estrogen level and bone loss. The effects of resveratrol on bone metabolism and bone turnover related indexes in ovariectomized rats were studied, hoping to provide a research basis for developing resveratrol to prevent and treat postmenopausal osteoporosis.

Materials and Methods

Experimental animals

48 clean grade healthy adult female unmated SD rats (about 3 months old, weight (220±18g)) were selected. Rats were purchased from the experimental animal department of XiangYa School of Medicine, Central South University. Animal number SCKK (Hunan) was 2018-0023. At the end of the experiment, SD rats were euthanized. Barbirate injection, including 4% pentobarbital, 20% urethane, and 1% chloral hydrate, was injected intraperitoneally (500 mg/kg) to anesthetize, and then KCL (124 mg/kg) was injected intravenously to sacrifice. The death of rats was verified by observation of indications such as breathing, heartbeat, pupil, and nerve reflex.

Main reagents and instruments

Resveratrol (purity 98%, purchased from Hunan Hongjiang Huaguang Biology Co., Ltd.); 17β-estradiol (Sigma); serum estrogen kit, tartrate-resistant acid phosphatase kit (Nanjing Jiancheng Bioengineering Institute); automatic biochemistry analyzer (Toshiba, Japan); BWJ-1 single-photon bone density detector (Jonchan Group's product).

Modeling and grouping

48 clean grade healthy adult female unmated SD rats were randomly divided into 6 groups, including normal control group (NCG), osteoporosis model group (OP MG), estrogen treatment group (17β-E2 group), RES low dose group (RES-L), RES medium-dose group (RES-M) and RES high dose group (RES-H). There were 8 animals in each group and the experiment began after 1 week of adaptive feeding. Rats in each group were anesthetized by intraperitoneal injection of 3.6% chloral hydrate (10 ml/kg) was injected intraperitoneally for anesthesia. Blood was collected from the heart and placed in a non-anticoagulant test tube. Blood samples were allowed to stand at 4°C for 4h, then centrifuged at low temperature, and upper serum was collected and stored at 20°C for testing. The levels of serum osteocalcin (BGP), alkaline phosphatase (ALP), tartrate-resistant acid phosphatase (TRAP), type I amino front-end peptide (PINP), type I collagen strong carboxyl peptide (CTX-I), urine deoxypyridinoline (DPD) and serum estrone were detected by enzyme-linked immunosorbent assay (ELISA).

Detection of indexes

Detection of calcium and phosphorus levels in serum and urine of rats in each group

The levels of serum calcium (S-Ca), serum phosphorus (S-P), urinary calcium (U-Ca/Cr), urinary phosphorus (U-P/Cr) were measured by automatic biochemistry analyzer. The U-Ca/Cr and U-P/Cr represent the amount of protein excreted when excreting 1g creatinine (Cr) and can be used as biochemical indexes for evaluating bone metabolism state of osteoporosis patients.

Serum biochemical indexes

Body weight was measured once every 4 weeks during modeling. At the end of the experiment, 3.6% chloral hydrate (10 ml/kg) was injected intraperitoneally for anesthesia. Blood was collected from the heart and placed in a non-anticoagulant test tube. Blood samples were allowed to stand at 4°C for 4h, then centrifuged at low temperature, and upper serum was collected and stored at 20°C for testing. The levels of serum osteocalcin (BGP), alkaline phosphatase (ALP), tartrate-resistant acid phosphatase (TRAP), type I amino front-end peptide (PINP), type I collagen strong carboxyl peptide (CTX-I), urine deoxypyridinoline (DPD) and serum estrone were detected by enzyme-linked immunosorbent assay (ELISA).

Detection of bone mineral density (BMD)

The bilateral femoral heads were quickly removed and the surrounding soft tissues were removed. The right femoral head was quickly placed in liquid nitrogen, then expelled and stored in a cryopreservation tube at -80°C for later use. The left femoral head was wrapped with saline gauze and stored in a cryopreservation tube at -80°C. The BMD of the distal metaphysis of the right femur of rats was measured by dual-energy x-ray absorptiometry.

Results

Serum estrogen levels of rats in each group

After treatment for three months, the serum estrogen levels of rats in each group were detected. The results showed that the serum estrogen levels in the OP group decreased significantly (P< 0.05) after operation for three months. After prophylactic treatment, serum estrogen levels in the 17β-E2 group, RES-M and RES-H treatment group increased compared with the OP group (P< 0.05), while those in the RES-L treatment group were not significantly increased (P=0.05). More details are shown in Figure 1.

The serum estrogen of the OP group was significantly lower than that of the control group after the operation. After prophylactic treatment, serum estrogen levels in the 17β-E2 group, RES-M and RES-H treatment group increased compared with those in the OP group (P<
significant difference in the expression levels of U-P and Cr between the 17β-E2 group, RES-L, RES-M, RES-H treatment group and healthy control group.

Detection of blood biochemical criterion related to bone metabolism of rats in each group

Detection by kit showed that the levels of TRAP, BGP, DPD and CTX-I in the OP group increased significantly (P< 0.05), while the levels of TRAP decreased significantly compared with those in the OP group (P< 0.05) after treatment with 17β-E2 and different doses of RES. The levels of PINP and ALP in OP MG increased significantly (P< 0.05), but the levels of PINP and ALP did not change significantly compared with those in NCG (P>0.05) after treatment with 17β-E2 and RES. IP and ALP increased in the middle and lower levels, with statistical difference compared with those in the OP group (P < 0.05). More details are shown in Figure 3.

Figure 3-A The expression levels of TRAP in the OP group were significantly higher than the healthy control group. There was no significant difference in the expression levels of TRAP between 17β-E2 group, RES-L, RES-M, RES-H treatment group and healthy control group. Figure 3-B The expression levels of BGP in the OP group were significantly higher than the healthy control group. There was no significant difference in the expression levels of BGP between 17β-E2 group, RES-L, RES-M, RES-H treatment group and healthy control group. Figure 3-C The expression levels of PINP in the OP group were significantly lower than the healthy control group. There was no significant difference in the expression levels of PINP between 17β-E2 group, RES-M, RES-H treatment group and healthy control group. However, the increase of the RES-L treatment group was not significant. Figure 3-D The expression levels of ALP in the OP group were significantly higher than the healthy control group. There was no significant difference in the expression levels of ALP between 17β-E2 group, RES-M, RES-H treatment group and healthy control group. However, the decrease of the RES-L treatment group was not significant. Figure 3-E The expression levels of DPD in the OP group were significantly higher than the healthy control group. There was no significant difference in the expression levels of DPD...
between 17β-E2 group, RES-L, RES-M, RES-H treatment group and healthy control group. Figure 3-F. The expression levels of CTX-I in the OP group were significantly higher than the healthy control group. There was no significant difference in the expression levels of CTX-I between 17β-E2 group, RES-L, RES-M, RES-H treatment group and healthy control group.

Detection of BMD of rats in each group

After 3 months of modeling treatment, the left femur of rats was taken. The BMD of the left middle femur and proximal metaphyseal of rats in each group were measured by dual-energy x-ray absorptiometry. The BMD of the OP group decreased significantly ($P<0.05$). The density in the 17β-E2 group, RES-M and RES-H increased and showed no statistical difference compared with NCG ($P>0.05$) after treatment with 17β-E2 and different doses of RES. There was no significant increase in BMD in the RES-L, which was statistically different compared with NCG ($P<0.05$). More details are shown in Figure 4.

The BMD of the OP group decreased significantly. The density in the 17β-E2 group, RES-M and RES-H increased and showed no statistical difference compared with those in NCG after treatment with 17β-E2 and different doses of RES. There was no significant increase in BMD in the RES-L, which was statistically different compared with those in NCG.

Discussion

Postmenopausal osteoporosis of women belongs to high convertibility osteoporosis. Due to the degeneration of ovarian function and estrogen deficiency, bone strength is damaged, BMD and bone quality are decreased, bone fragility is increased and fracture is easy, which seriously affects the physical health and quality of life of middle-aged and elderly women, and even shortens their life span (8). The study of OP in modern medicine has reached the molecular level. It is believed that the basic pathological mechanism of OP is the clinical symptoms caused by an imbalance of coupled response of bone absorption and bone formation in the process of bone metabolism, resulting in an imbalance of calcium and phosphorus metabolism in the human body and gradual decrease of bone density (9,10). Ovariectomized rats are classic models for studying the pathogenesis of PMOP and observing the efficacy of drugs.

In this experiment, the ovariectomized osteoporosis rat model was used to simulate the development of postmenopausal osteoporosis. After 3 months of modeling, compared with those in NCG, serum estrogen levels decreased, TRAP and ALP increased and bone metabolism was in a high transition state in the OP MG (11, 12). 50% of ALP in serum comes from the bone. The activity of ALP can reflect the activator of osteoblasts, especially newly formed osteoblasts. The inhibition of osteoclasts is released during the period of estrogen reduction or deficiency and bone absorption increases significantly, with bone formation also increasing. That is, high conversion OP also brings ALP value higher. Biochemical indexes of blood and urine can reflect bone metabolism levels. After ovariectomy, it will cause calcium and phosphorus metabolism disorder, decrease blood calcium and bone calcium, increase urine calcium discharged by the kidney and cause calcium deficiency. The increase of blood phosphorus and urine phosphorus also reduces the capacity of bone formation and bone calcification, resulting in a decrease of mineral deposition, lack of bone material base, reduction of BMD and bone mass, increase of bone fragility and lead to osteoporosis and even fracture (13, 14). In addition, it can also cause an increase in bone turnover rate after ovariectomy. Bone resorption is greater than bone formation, which hinders the increase of bone mass (15). In this experiment, it was found that after intervention with resveratrol, the blood calcium and serum phosphorus levels increased, urine calcium and urinary phosphorus levels decreased, calcium and phosphorus metabolism improved and bone tissue calcification strengthened in ovariectomized rats. At the same time, compared with OP MG, serum estrogen levels, TRAP, ALP and other indicators have significant differences in the 17β-E2 group and different doses of RES treatment group. It approached the NCG, indicating that RES treatment has an estrogen-like effect and can effectively prevent osteoporosis after ovariectomy (16).

Osteocalcin and collagen are the main proteins constituting bone tissue (17). Serum PINP and OCN are biochemical indexes of bone formation (18). CTX-I and urine DPD are biochemical indexes of bone resorption (19). In the study, it was found that the bone turnover rate of ovariectomized rats increased, bone formation and bone absorption increased, and the amount of PINP decreased significantly. The amounts of OCN, CTX-I, ALP and DPD increased significantly (20,21). In this experimental study, it was also found that resveratrol can increase the amount of PINP in the blood of ovariectomized rats and reduce the amounts of ALP, CTX-I, OCN and DPD, thus regulating the metabolism of bone collagen and non-collagen, reducing the high bone turnover rate induced by ovariectomy; promoting bone formation and inhibiting bone absorption (22-39). Genome editing (40) can be very effective in this regard.

In conclusion, in vivo experiments were used to prove that after ovariectomy, the levels of estrogen in adult female SD rats decreased, the expression and function of antioxidant enzymes in bone tissue decreased, the bone tissue was in the oxidative stress, the number and function of osteoblasts and osteoclasts were
unbalanced, bone absorption was greater than bone formation, bone metabolism was unbalanced, and finally lead to the development of osteoporosis. Resveratrol has an estrogen-like effect, which can antagonize the pathological process of osteoporosis and play a role in protecting bone tissue. This study provides a theoretical basis for the mechanism of RES in the treatment of osteoporosis.

References


