· Original article ·

The effect of the phytoestrogen genistein on metabolism of bones in ovariectomy rats and IL-6 in celiac macrophages of mice

SUN Ji-yuan(孙纪元) LV Yong-gang(吕勇刚) LI Ji-peng(李纪鹏) MIAO Shan(缪珊)
WANG Si-wang(王四旺) JIE Qiang(颉强) XIE Yan-hua(谢艳华) WANG Jian-bo(王剑波)
YANG Qian(杨倩)

【**Abstract**】 **Objective** Genistein, as a phytoestrogen, is a type of soybean-derived isoflavone that possesses structural similarity to become estrogen. The purpose of this study was to elucidate whether the administration of genistein, extracted from a Chinese herbal medicine *Huaijiao* (*Sophora japonica-Leguminosae*), is capable of preventing rapid bone loss occurring in rats after surgical ovariectomy and improving the symptom caused by this. **Methods** Sixty rats were randomly divided into six groups, including control (sham-operated), ovariectomized model (OVX), E2-treated (E2) group that were subcutaneously injected with E2 at a dosage of 1.5 mg/kg once a week, and genistein-treated (Gen) groups at the dosage of 4.5 mg/kg, 9.0 mg/kg and 18 mg/kg respectively. The treatment was administrated 8 days later after the operation. Samples were taken from every group randomly at 12 weeks after the treatment. Parameters, such as body weight, serum biochemical criterion, bone mineral density (BMD) and biomechanics, were evaluated. **Results** The results indicated that genistein could restrain body weight, increase the levels of serum Ca, Mg, P, calcitonin(CT) and decrease the levels of bone gla protein (BGP) and alkaline phosphatase (ALP) significantly. Compared with OVX group, genistein at the dosage of 4.5 mg/kg and 9 mg/kg enhanced the BMD of femur, tibias and L2-4. Genistein could also meliorate biomechanical indexes of rats ($P<0.05$ or $P<0.01$). **Conclusions** In comparison with the anti-osteoporosis effect of E2, the genistein extracted from *Huaijiao* has the same beneficial effect on anti-osteoporosis and has little side effect.

【**Key words**】 Genistein; Bone mineral density; Biomechanics; Osteoporosis; Ovariectomy; Serum biochemical criterion

Introduction

Osteoporosis is a metabolic disease of the bone and increases the likelihood of bone fracture. Decreased bone density is a major risk factor of osteoporosis[1-2]. The generalized loss of bones, the development of osteoporosis and the occurrence of fractures all increase with ages. It is anticipated that the cost for the hip fracture therapy in the United States will approach 250 billion US dollars by 2050. Whereas bone mineral density (BMD) declines in both men and women with ages, women typically start with lower BMDs and show an accelerated loss at menopause owing to a decline in estrogen production by ovarian hormone deficiency[3-4]. It is well-known that estrogen deficiency in postmenopause and ovariectomy leads to the acceleration of bone resorption and the results in rapid bone loss with a high bone metabolic turnover, increasingly developing to osteoporosis[5]. Moreover, the occurrence of osteoporosis is associated with a large increase in osteoclast numbers caused by enhanced osteoclast formation and reduced

DOI:10.3877/cma.j.issn.1674-0785.2011.20.042

Institute of Material Medical, School of Pharmacy(SUN Ji-yuan, MIAO Shan, WANG Si-wang, XIE Yan-hua, WANG Jian-bo, YANG Qian), Department of Gastrointestinal Surgery(LV Yong-gang, LI Ji-peng), Department of Orthopaedics Surgery(JIE Qiang), Xijing Hospital, Fourth Military Medical University, Xi'an 710033, China

Corresponding author: WANG Si-wang, Email: wangsiw@fmmu.edu.cn

osteoblast activity. Current therapies recommended for postmenopausal osteoporosis treatment include the supplementation with estrogen or hormone replacement therapies (ERT or HRT), calcitonin, bisphosphonates and raloxifene[6]. Estrogen is the most potent inhibitor of bone resorption and the most widely recommended therapy to reduce the rate of postmenopausal bone loss. However, the available evidence appears to suggest that the long-term use of ERT has numerous side effects (e. g., uterine bleeding and hyperplasia, cardiovascular disease, gall bladder disease, estrogen induced endometrial cancer and an increasing risk of breast cancer)[7-8]. By 352 cases postmenopausal women, Hua et al[9] find bone quantity loss of the skeleton was more quick, and should been intervene with clinic medical method more early at menopause or postmenopausal women. Compared with white women, the low incidence of osteoporosis and heart diseases in postmenopausal Asian women has been associated with their high intake of soy foods containing abundant of genistein. Currently, natural alternatives to estrogen with estrogen like activities such as genistein (Gen) prevents bone loss associated with estrogen loss in postmenopausal women[10] and experimentally in ovariectomized (OVX) animals without estrogenic effects on the uterus[11-12].

Materials and Methods

1. Reagents

Genistein (purity≥98.5%; Batch No. 001020) was provided by Institute of Material Medical, School of Pharmacy, Fourth Military Medical University, Xi′an, China. It was extracted from *Huaijiao* and analyzed with IR, UV, HNMR and CNMR. The molecular formula of the compound is C15H10O5 with a structure as below: The structure was identical to those reports for genistein[13] (Figure 1). E2(17-estradiol; Batch No. 060517), as a positive control to anti-osteoporotic effect, was manufactured by Hualian Pharmaceuticals CO. LTD., Shanghai, China.

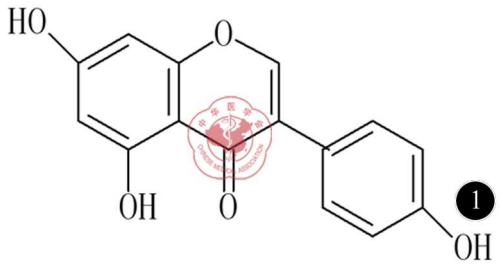

Figure 1 The structure of genistein

2. Animals

60 three-month-old female Sprague-Dawley(SD) rats weighing approximately 240 g were purchased from Experimental Animal Research Center of Fourth Military Medical University (certification No. 08-013; Xi′an, China). The rats were housed in a room which provided alternating 12 h periods of light and dark with the room temperature at (23±1)℃ and humidity(55±5)%. All animals were allowed free access to distilled water and fed on a commercial diet. All animals were weighed weekly during the experimental period. Animal care and use conformed to the guide to the Care and Use of Experimental Animals, and the University of Fourth Military Medical University Animal Care Committee approved the experimental protocol.

3. Experimental design

The SD rats were sham-operated or ovariectomy operated within days of arrival. The surgery procedure performed as follows. The animals were anesthetized with 1% sodium barbital and fastened abdomenlaterally. The hair of the operative area was disinfected with 70% ethanol, and a dorsal midline incision was made through the skin at the level of the kidneys. The exposed ovaries through the thin muscle wall by retracting the skin laterally toward either side were pulled into the incision and excision after the ligation of the upper horn of the uterus. The wounds were closed with surgical clips. The same procedure was applied to sham controls except for the ligation of the upper horn of the uterus and the excision of the ovaries. The animals were then randomly divided into 6 groups: sham-oper-

ated control group (Sham), ovariectomized group (OVX), OVX treated with E2 (subcutaneous injection at the dosage of 1.5 mg/kg once a week), and OVX treated with genistein at 4.5 mg/kg(GenL), 9 mg/kg(GenM) and 18 mg/kg(GenH) respectively by intra gastro-intestinal (IG) daily. The treatment was administrated 8 days later after the operation. Samples were taken from every group randomly at 12 weeks after the treatment. The wet weight of uterus was measured when all rats were sacrificial.

4. BMD of the femur, tibia and lumbar vertebrae 2-4(L2-4)

Femora, tibias and L2-4 were immediately removed and stored at −20 ℃ until analyses of BMD were performed. They were analyzed by dual-energy X-ray absorptiometry(DEXA; Lunar Co. USA, Software Version 1.0C) at the Centre for Bone Research. Femora, tibias and vertebrae were scanned as previously described.

5. The parameters of whole femur

Mensuration of bone mass includes dry weight, ash weight, calcium of the whole femur. The femur of posterior limb cleans soft tissue, bakes the femur to constant weight under 105 ℃, then measures the dry weight of femur with exquisite analytic balance and the ash weight of it under 550 ℃ for 6 hours.

6. Blood and urine biochemical index

The blood biochemical indexes including of Ca, P, Mg, alanine aminotransferase (ALT), alkaline phosphatase (ALP), bone gla protein (BGP) and calcitonin (CT) were determined.

Ca, P, Mg with the endpoint method, ALT and ALP with the enzyme motive power method, BGP and CT with the radio-immunity method.

Urine calcium was determined with orthocresol phthalein complexation chromatometry, urine hypoxanthine alkalinity trinitrophenol, urinary hydroxyproline with the method of chloramine T oxidization chromatometry.

7. Three-point bending test at midpoint of femora

Three-point bending at the midpoint of the femur was performed to determine the biomechanical strength properties of a skeletal site enriching in cortical bones by using a computer-controlled mechanical testing machine (INSTRON 1195, England) equipped with a 500 N M-SI sensor (Celtron). The bones were thawed at a room temperature, and the saline solution was regularly applied to the specimens to prevent drying. In preparation for testing, the posterior sides of the femur were placed on two base supports of a bending jig separated by 5 mm, with the midpoint directly under the crosshead. The crosshead was lowered at a speed of 2 mm/min until the fracture occurred. The bending jig is designed to minimize shear forces during testing. These tests have been previously described in details [14]. From the load-displacement curve that was generated, yield load, resilience, ultimate stiffness, peak load, and rigidity were determined. Yield load, resilience, and ultimate stiffness primarily measure the contribution of mineral to bone strength, while peak load and rigidity primarily measure the contribution of matrix to bone strength. Yield load is the load at which the curve of the load-displacement curve becomes non-linear (designating the point at which permanent damage to the bone will start to occur), resilience is the energy absorbed by the bone up to the yield load, and stiffness is a measure of the extrinsic rigidity of the bone. Peak load is the maximum force a bone withstands before it fractures, and rigidity is the energy the bone absorbs until it fractures.

8. Specimens

Three sections for each animal (both sides in the same section) were analyzed without knowledge of group or day using a light microscope and a digital camera interfaced with Sigma Scan Pro 5 software (SPSS Inc). The distance between the bone and the cellular infiltration nearest to the bone was measured.

9. Double fluorescent staining

All the animals were double-labeled with subcutaneous injections of tetracycline (30 mg/kg BW) and calcein (5 mg/kg BW) at 10 days and 3 days before sacrificed.

10. Statistics

Data is expressed as means ± *SD*, and the differences among treatments were determined by one-way ANOVA coupled with the Duncan's multiple range test. Differences with $P<0.05$ were considered statistically significant.

Results

1. Weight of body, uterus, and the BMD of femur, tibia and L2-4

The body weight of the OVX rats were increased at 12 weeks after operation, but it had no statistical significance with other groups. The uterine wet weight increased significantly in E2 groups (versus OVX rats, $P<0.01$). After administrated with genistein for 12 weeks, genistein could increase the uterine wet weight ($P<0.01$ or $P<0.05$, Table 1).

Table 1 Effect of genistein on body weight, left femur weight and left tibia weight of rats ovariotomized the whole femur bone mass and dimensions; and biomechanical strength properties at femur midpoint ($\bar{x}\pm s, n=10$)

Group	Body weight (g)	Uterine weight (mg)	BMD (mg/cm²)			Whole femur		
			Femur	Tibia	L2-4	Dry weight (mg)	Ash weight (mg)	Calcium content (mmol/g)
Sham	302.8 ± 37.0	588.6 ± 63.3	0.14 ± 0.01	0.12 ± 0.01	0.16 ± 0.02	687.9 ± 37.0	381.0 ± 37.5	4.69 ± 0.30
OVX	332.4 ± 48.5	268.9 ± 59.9[b]	0.13 ± 0.01[a]	0.10 ± 0.02[a]	0.13 ± 0.01[b]	648.1 ± 30.9[a]	346.6 ± 31.4[a]	4.34 ± 0.30[a]
E2	262.4 ± 39.1	436.3 ± 73.6[d]	0.15 ± 0.01[bd]	0.13 ± 0.01[d]	0.15 ± 0.01[d]	678.6 ± 32.7[c]	379.3 ± 36.3[c]	4.67 ± 0.33[c]
GenL	312.1 ± 33.4	366.3 ± 52.8[d]	0.15 ± 0.01[bd]	0.13 ± 0.01[d]	0.15 ± 0.02[c]	684.2 ± 30.1[c]	380.4 ± 34.8[c]	4.75 ± 0.42[c]
GenM	323.8 ± 31.6	395.4 ± 56.0[d]	0.14 ± 0.01[bd]	0.12 ± 0.01[d]	0.15 ± 0.02[c]	683.9 ± 40.4[c]	381.6 ± 39.0[c]	4.83 ± 0.31[d]
GenH	321.8 ± 35.5	296.0 ± 63.0[c]	0.14 ± 0.01[c]	0.12 ± 0.01[c]	0.14 ± 0.01[a]	680.7 ± 59.0	372.7 ± 36.2	4.75 ± 0.55

Group	Femur midpoint				
	Yield load (N)	Resilience (N/mm)	Ultimate stiffness (N/mm)	Peak load (N)	Rigidity (N/mm)
Sham	28.17 ± 1.40	1.37 ± 0.07	419.80 ± 22.34	147.2 ± 14.1	4.30 ± 0.35
OVX	21.53 ± 1.99[a]	0.98 ± 0.11[b]	345.50 ± 28.78[b]	109.2 ± 16.2[b]	3.67 ± 0.52[b]
E2	24.88 ± 1.80[c]	1.25 ± 0.18	399.50 ± 16.20[d]	137.9 ± 14.9[d]	3.97 ± 0.31[c]
GenL	26.85 ± 1.30[c]	1.30 ± 0.14[c]	388.45 ± 26.12[c]	141.2 ± 17.3[d]	4.11 ± 0.26[c]
GenM	29.15 ± 2.16[c]	1.42 ± 0.12[d]	412.80 ± 27.38[d]	144.7 ± 17.7[d]	4.60 ± 0.55[d]
GenH	25.43 ± 4.12[c]	1.38 ± 0.19[c]	394.30 ± 23.43[c]	141.4 ± 15.1[d]	4.25 ± 0.44[c]

Group	Blood biochemical							Urine biochemical	
	Ca (mmol/L)	P (mmol/L)	Mg (mmol/L)	ALT (U/L)	BGP (mmol/L)	CT (mmol/L)	ALP (mmol/L)	Ca/Cr	Pyd/Cr
Sham	2.24 ± 0.27	1.94 ± 0.23	1.48 ± 0.38	158.7 ± 48.6	5.06 ± 0.78	54.69 ± 12.78	236.8 ± 41.2	2.23 ± 0.31	0.13 ± 0.01
OVX	1.93 ± 0.29[a]	1.60 ± 0.34[a]	1.01 ± 0.24[a]	243.5 ± 34.9[b]	6.03 ± 0.70[a]	42.21 ± 6.12[a]	290.6 ± 68.6[a]	2.62 ± 0.25[b]	0.15 ± 0.02[b]
E2	2.24 ± 0.32[c]	1.85 ± 0.33	1.12 ± 0.46	123.1 ± 59.7[d]	5.73 ± 0.83	52.74 ± 13.60	218.7 ± 68.8[c]	2.40 ± 0.19[c]	0.14 ± 0.01[c]
GenL	2.31 ± 0.36[c]	2.05 ± 0.36[c]	1.12 ± 0.32	204.4 ± 61.7[d]	5.27 ± 0.60[c]	52.90 ± 10.81[c]	198.6 ± 58.6[d]	2.39 ± 0.22[c]	0.13 ± 0.02[c]
GenM	2.39 ± 0.34[c]	1.94 ± 0.11[c]	1.35 ± 0.33[c]	181.6 ± 58.8[d]	5.27 ± 0.67[c]	56.49 ± 13.15[c]	211.3 ± 63.3[c]	2.36 ± 0.24[c]	0.13 ± 0.01[d]
GenH	2.28 ± 0.32[c]	2.06 ± 0.21[d]	1.11 ± 0.37	224.2 ± 77.9[a]	5.64 ± 0.75	54.20 ± 7.12[d]	194.1 ± 30.8[ad]	2.33 ± 0.28[c]	0.13 ± 0.02[c]

Gen = genistein; L: 4.5 mg/kg genistein; M: 9 mg/kg genistein; H: 18 mg/kg genistein. Compared with the sham group, [a]$P<0.05$, [b]$P<0.01$; Compared with OVX group, [c]$P<0.05$, [d]$P<0.01$

Compared with Sham group, the BMD of femur, tibia and L2-4 in OVX degraded and had the statistical significance. When treated with genistein or E2 for 12 weeks, the BMD of the above-mentioned bone increased, especially of genistein at the dosage of 4.5 mg/kg and 9 mg/kg.

2. Effect of genistein on the whole femur

Compared with Sham group, the dry weight, ash weight, calcium content of the whole femoral bone in OVX rats

were significantly lower. When treated with genistein or E2, the parameters of the femur became higher and the effect of low dose genistein and middle dose genistein was evident ($P<0.01$ or $P<0.05$, Table 1).

3. Blood biochemical index

Compared with Sham group, the serum level of calcium, phosphorus and magnesium were significantly lower, while the level of serum ALT, BGP, ALP was significantly higher. After being given the genistein, the level of serum phosphorus of rats was significantly higher than that in OVX group.

4. Bone biomechanical assessment

The results showed that genistein at the dosage of 9 mg/kg and 18 mg/kg could improve extrinsic biomechanical properties at the mid-diaphyseal region of the left femur. The 3-point bending test revealed a significant and positive effect of genistein on resilience, yield load and peak load variables (Table 1) also positively influenced ultimate stiffness and rigidity ($P<0.05$) compared with the Sham group.

5. Double fluorescent staining

Pathophotograph display that duplicate band florescence of microspecimen of femur, one is the cefracycline showed green yellow and the other is calcein showed green by fluorescent microscope. The slice of tibia in Sham group showed two lines of fluorescent extreme proximity, the line of clear fluorescence. The interval between the two lines greatens and the lines are vague relatively in OVX rats. Treatment with the different dosage of genistein lasted for 12 weeks, two lines of fluorescent were proximal gradually, and the line of fluorescence turned clearer than that of OVX group (Figure 2).

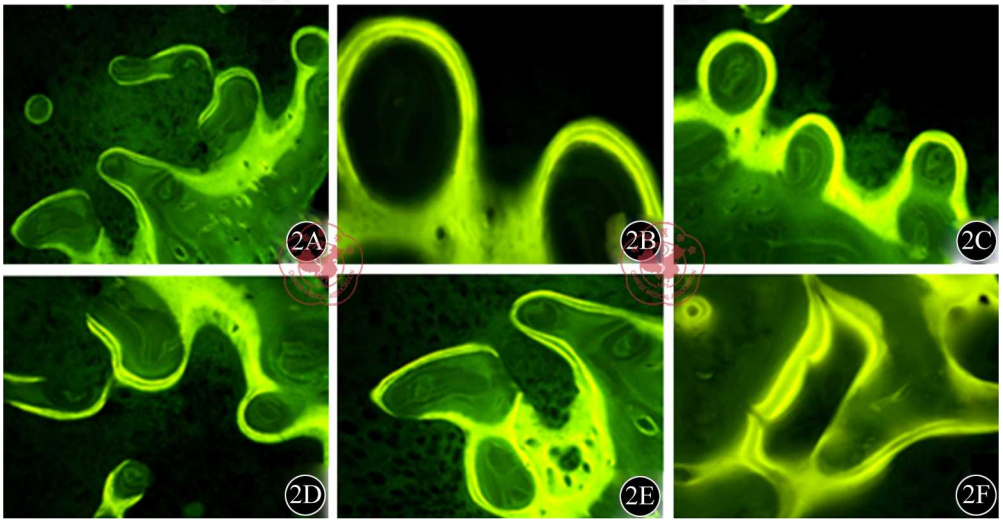

Figure 2 Histological analysis of the trabecular bone collected from Sham rats(2A), OVX group(2B), OVX rats treated with E2(2C) or genistein (2D represents GenL; 2E represents GenM; 2F represents GenH). Rats were sham-operated or OVX and some of the OVX rats were treated with 4.5 mg/kg, 9.0 mg/kg or 18 mg/kg of genistein or subcutaneous injection 1.5 mg/kg of E2 once a week. The femora were collected 12 weeks after the operation, and the sections of distal metaphysis were prepared(2A). Sections of the trabecular bone were stained for TRAP (original magnification)(2B). Two-dimensional histomorphometric parameters of the trabecular bone were shown in (2A) (×400)

Discussion

In general, the bone-protective effect of ERT in PMOP has been recognized. However, the side effect induced by administering estrogen limits its clinical application. Previous studies have shown that the phytoestrogen genistein effectively inhibited bone loss without inducing adverse effects such as uterine hypertrophy and endometrial hyperplasia. However, the cellular and molecular mechanism by which phytoestrogen regulates bone metabolism remains

unclear.

Genistein, a major isoflavone phytochemical in some plants belonging to the Leguminosae family, is known as a phytoestrogen that is capable of binding to the estrogen receptor. Much attention has been focused on the role of genistein in preventing bone loss resulted at least in part from the estrogen deficiency. Furthermore, the finding that genistein acts as a selective estrogen receptor modulator (SERM) on osteoblastic cells and in OVX rats highlights the possibility that this isoflavone phytochemistry hardly affects the uterus in some dose ranges at which it does exert the estrogen-like regulative action on bone and bone marrow[15].

Osteoporosis model induced by ovariotomy rats, simulates exactly the clinical characteristics of adult women on hormonoprivia and the reaction of estrogen replacement therapy. Osteoporosis process of rats′ cancellous bone is very advantageous to study the physiological reaction of bone. To study the prevention and restoration of the estrogen-deficient osseous loss, researchers have enough time to observe for rats. A large number of previous reports on ovariectomized rodents have revealed that genistein and E2 treatment significantly increased the BMD and remarkably improved the bone biomechanical performance and microstructure. A possible explanation for this is that although rodent models following ovariectomy lacked estrogen, the ER-OPG route remained intact. Furthermore, it is presumed that genistein and E2 stimulate OPG expression by activating the ER and inhibiting bone resorption in order to exert inhibitive effect on bone loss. However, this assumption has not yet been confirmed. OPG gene knockout mice provide a model for observing the functions of this gene *in vivo*. These mice exhibit progressive intensive high-turnover bone loss and display characteristics of normal multiplication and growth; therefore, this animal model is ideal for investigating whether genistein regulates bone metabolism depending on the OPG pathway *in vivo*[16].

The present study demonstrates the beneficial effect of systemically administered a large-scale purified genistein from *Huaijiao* could prevent osteoporosis in many criteria including bone density, bone mineral components such as calcium, phosphorus, and magnesium in the OVX rats. The analytical measurement by IR, UV, HNMR and CNMR methods indicates the chemical structure of genistein from *Huaijiao* is 5,7,4′-trihydroxyisoflavone, which is similar to those commercial available genistein[1]. Our findings on genistein from *Huaijiao* are consistent with other reports using genistein from other sources that bone loss in short-trem OVX rats was significantly recovered by genistein dosage[17]. Ohta et al[18] reported that isoflavones increased BMD in intact mature mice.

In our experimental model, the bone densities of femur and tibia as well as trabecular thickness, area percentage and numbers in OVX rats decreased, and such osteoporosis effect is statistically significant in comparison to the control group up to 4 weeks. The effective dosages of genistein from *Huaijiao* on bone loss in OVX rats were 4.5 mg/kg and 9 mg/kg of body weight. Higher dosage (18 mg/kg) of genistein from *Huaijiao* had somewhat less positive effect on preventing bone loss. Our results are comparable to the effectiveness of purified genistein from soybean where the maxima effective dosage is 5 mg/kg of body weight. Five-fold higher dosage (25 mg/kg) has less pronounced anti-osteoporosis effect on OVX rats[19-20]. Genistein and daidzein, are known as phytoestrogens because they are chemicals found in plants that bind to the estrogen receptor. Now, generally believed that effect of prevention osteoporsis will decrease when estrogen receptor saturated.

The bone mineral components including calcium, phosphorus, and magnesium also dropped off in OVX model rats and to the maximum decrease at 12 weeks after ovariectomizing. The loss of mineral contents in the OVX animals is also statically significant. Treatment of genistein from *Huaijiao* with low (4.5 mg/kg) and medium (9 mg/kg) dosages on OVX rats has 2-fold more effectiveness on calcium recovery than that with high dosages (18 mg/kg) and E2. The pharmacological effect on bone magnesium and phosphorus contents recovery was no dramatic differences among the E2 and three-dosages of genistein. This preventive effect of genistein from *Huaijiao* on bone calcium loss of OVX rats is comparable to those treated with genistein from soybean[21].

In our trial on suppressed osteoporosis with genistein from *Huaijiao* with a newly state-of-art purification proce-

dure, we demonstrate that genistein from *Huaijiao*, which is the same as genistein from natural plants, prevents bone loss in an OVX rate model of osteoporosis [5]. Additionally, its efficacy in effective dosage is comparable to genistein from other sources. Therefore, *Huaijiao* could potentially be a valuable natural plant to purify genistein in the future.

Main components of plant medicine *Huaijiao* have much obviously effect on prevention and cure cancer, cut down hypolipemia, promote phytolectin form, elevation micrangium resistance, anti oxydation and free radical, restrain lipoxygenase haemolysis, promotion Ca, Mg, P deposition in animal bones.

Administration of ovariectomized animals with contained synthesis or natural isoflavone forage could prevent the decrease caused by estrogen-deficient such as cholesterol changes in the liver and serum. Isoflavones have surely prevention and treatment for women when they are in menopause time with many diseases related to hormone fall-off such as osteoporosis, atherosis and high blood fat. The experiment results of Nakajima et al [2] and Wu et al [12] showed that genistein at the dose of 12 mg/kg, bonding with the training, could prevent and treat the sclerotin loss of femoral bone and lumbar efficiently caused by spaying the rats. Ishimi et al [5] confirmed that genistein at the dosage of 0.7 mg/d-3.5 mg/kg by subcutaneously could prevent and treat the trabecular bone loss of ovariectomized rats, and there is no apparently effect on preventing the bone mineral loss with the high dose group (5 mg/d≈25 mg/kg). Study by Nogowski et al [11] indicated that genistein in the food could notablely relieve the content of TG in the muscle and serum, FFA, and lipidic metabolism. Ovariectomized rats administrated with genistein at different dosage of 0.5, 1.6 or 5.0 mg/d respectively by Anderson et al [22] showed that low and middle dose group could make the ovariectomized rats BMD of cancellous bone improved obviously, by scanning electron microscopes he also finds that the numbers and the density of tibia bone trabecular, and significant difference in high dose group according to our experiment results.

A large number of previous reports on ovariectomized rodents have revealed that genistein and E 2 treatment significantly increased the BMD and remarkably improved the bone biomechanical performance and microstructure. A possible explanation for this is that although rodent models following ovariectomy lacked estrogen, the ER-OPG route remained intact. Furthermore, it is presumed that genistein and E2 stimulate OPG expression by activating the ER and inhibiting bone resorption in order to exert inhibitive effect on bone loss. However, this assumption has not yet been confirmed.

A previous *in vitro* study demonstrated that genistein could increase ALP activity in mice [23]. Further, *in vivo* studies, it revealed that genistein may increase the ALP levels in postmenopausal women [24] and in ovariectomized rats and that it could significantly decrease TRAP-positive cell formation in the rat tibia marrow and thus remarkably reduce the number of osteoclasts [25]; these results indicated that genistein could achieve some regulatory effect on both osteoblasts and osteoclasts.

Acknowledgements The authors have no conflict of interest of any kind related to the work presented in this publication. The authors are greatly thankful to Prof. LI Yun-xin (Dept of Engineering Mechanics, Xi'an Jiaotong University) for his technical assistance and special thanks given to LI Xiao-juan for her measuring the BMD. This study was supported by Technology Department, Shaanxi Province

(本文中文文题为:植物雌激素染料木素对去卵巢大鼠的骨代谢的影响)

References

[1] Wang ZL, Sun JY, Wang DN, et al. Phyto- medicine. Pharmacological studies of the large-scaled purified genistein from Huaijiao (Sophora japonica-Leguminosae) on anti-osteoporosis. Phytomedicine, 2006, 13: 718-723.

[2] Nakajima D, Kim CS, Oh TW, et al. Suppressive effects of genistein dosage and resistance exercise on bone loss in ovariectomized mice. J Physiol Anthropol Appl Human Sci, 2001, 20: 285-291.

[3] Toshiyuki H, Tsuneko O. Relationship between urinary phyto- estrogen levels and Z score of lumbar vertebral bone mineral density in Japanese

postmenopausal women. Nutrition Research,2006: 409-412.

[4] Atteritano M,Pernice F,Mazzaferro S,et al. Effects of phytoestrogen genistein on cytogenetic biomarkers in postmenopausal women: 1 year randomized,placebo-controlled study. Eur J Pharmacol,2008,589: 22-26.

[5] Ishimi Y,Arai N,Wang X,et al. Difference in effective dosage of genistein on bone and uterus in ovariectomized mice. Biochem Biophys Res Commun,2000,274:697-701.

[6] Tian Z,Wen M,Wang Z,et al. The preparation of genistein and LC-MS/MS on-line analysis. Drug Dev Res,2004,61:6-12.

[7] Almeida OP,Lautenschlager NT,Vasikaran S, et al. A 20-week randomized controlled trial of estradiol replacement therapy for women aged 70 years and older: Effect on mood,cognition and quality of life. Neurobiology of Aging,2006,27:141-149.

[8] The Writing Group for the 3rd European Conference on Sex Steroids and Cardiovascular Diseases,2003. The European Consensus Development Conference 2002: Sex Steroids and Cardiovascular Diseases: On the route to combined evidence from OC and HRT/ERT. Maturitas,44: 69-82.

[9] Hua JM,Dong QR,Shen YX,et al. A clinical investigation on postmenopausal spine osteoporosis. Chin J Clinicians(Electronic Edition),2009,3: 797-803.

[10] D'Anna R,Cannata ML,Marini H,et al. Effects of the phytoestrogen genistein on hot flushes,endometrium,and vaginal epithelium in postmenopausal women: a 2-year randomized,double-blind,placebo-controlled study. Menopause, 2009,16:301-306.

[11] Nogowski L,Mackowiak P,Kandulska K,et al. Genistein-induced changes in lipid etabolism of ovariectomized rats J. Ann Nutr Metab,1998,42: 360-366.

[12] Wu J, Wang XW, Takasaki M. Cooperative effects of exercise training and genistein administration on bone mass in ovariectomized mice. J Bone Miner Res,2001,16: 1829-1836.

[13] Cheng BL,Yegge JA,Bailey DT,et al. Process for isolation and purification of isoflavones,US,1997,Patent#5679806.

[14] Fonseca D,Ward WE. Daidzein together with high calcium preserve bone mass and biomechanical strength at multiple sites in ovariectomized mice. Bone,2004,35:489-497.

[15] Frost HM,Jee WS. On the rat model of human osteopenias and osteoporoses. Bone Miner,1992,18:227-236.

[16] Bucay N, Sarosi I, Dunstan CR, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes,1998, 12:1260-1268.

[17] Power KA, Ward WE, Chen JM, et al. Genistein alone and in combination with the mammalian lignans enterolactone and enterodiol induce estrogenic effects on bone and uterus in a postmenopausal breast cancer mouse model. Bone, 2006,39: 117-124.

[18] Ohta A,Uehara M,Sakai K,et al. A combination of dietary fructooligosaccharides and isoflavone conjugates increases femoral bone mineral density and equal production in ovariectomized mice. J Nutr,2002,132: 2048-2054.

[19] Fanti P,Monier-Faugere MC,Geng Z. The phytoestrogen genistein reduces bone loss in short-term ovariectomized rats. Osteoporos Int,1998,8: 274-281.

[20] Pie JE,Park JH,Park YH,et al. Effect of genistein on the expression of bone metabolism genes in ovariectomized mice using a cDNA microarray. J Nutr Biochem,2006,17:157-164.

[21] Lee YB,Lee HJ,Kim KS,et al. Evaluation of the preventive effect of isoflavone extract on bone loss in ovariectomized rats. Biosci Biotechnol Biochem,2004,68:1040-1045.

[22] Anderson JJ, Ambrose WW, Garner SC. Biphasic effects of genistein on bone tissue in the ovariectomized,lactating rat model. Proc Soc Exp Biol Med,1998,217:345-350.

[23] Li B,Yu S. Genistein prevents bone resorption diseases by inhibiting bone resorption and stimulating bone formation. Biol Pharm Bull,2003,26: 780-786.

[24] Morabito N,Crisafulli A,Vergara C,et al. Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study. J Bone Miner Res,2002,17: 1904-1912.

[25] Gao YH,Yamagughi M. Suppressive effect of genistein on rat osteoclast apoptosis is induced through Ca^{2+} signaling. Biol Pharm Bull,1999,22: 805-809.

(Received:2011-09-25)

(Editor: ZHANG Lan)

SUN Ji-yuan,LV Yong-gang ,LI Ji-peng,et al. The effect of the phytoestrogen genistein on metabolism of bones in ovariectomy rats and IL-6 in celiac macrophages of mice[J/CD]. 中华临床医师杂志:电子版,2011,5(20):6057-6064.