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Original Research Article

Effect of quercetin on bone metabolism and serum osteocalcin in osteoporotic rats

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Abstract

Purpose: *To determine the effect of quercetin on bone metabolism and serum osteocalcin in osteoporotic rats.*
Methods: *Sixty specific pathogen-free rats were randomly divided into control group, model group; high, medium and low dose quercetin groups, and diethylstilbestrol group, with 10 rats in each group. The high, middle and low dose quercetin groups were given quercetin suspension at doses of 200, 100, 50 mg/kg/day, respectively; the ethylene estradiol group was given ethylene estradiol (1.0 mg/kg/week), while control rats received ethylene estradiol at doses of 200, 100, 50 mg/kg/day. Rats in the model group were given saline. Samples were taken after 6 weeks of administration. The levels of serum bone-derived alkaline phosphatase (BALP), estradiol (E2) and serum osteocalcin (BGP) in femur tissue were measured using ELISA kits. Bone mineral density (BMD) was determined using BMD tester.*
Results: *Relative to normal rats, BALP and BGP levels in the model rats were markedly increased, while E2 was significantly lower ($p < 0.5$). Quercetin treatment led to significant increases in BALP and E2 levels in the middle and high dose groups, relative to the model group, while BGP levels in all quercetin treatment groups decreased significantly, when compared to model rats ($p < 0.05$). There were higher BMD values in quercetin and diethylstilbestrol groups than in model ($p < 0.05$).*
Conclusion: *Quercetin enhances bone formation and BMD, but decreases osteocalcin levels and maintains bone biomechanics in ovariectomized rats. Thus, it may find therapeutic application in maintaining bone health.*

Keywords: *Quercetin, Osteoporosis, Bone metabolism, Osteocalcin*

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INTRODUCTION

Osteoporosis (OP), a metabolic disease of the bone, is characterized by absolute reduction in bone mass and degradation of bone tissue, resulting in increased bone fragility and even fracture. The early symptoms of the disease are atypical, and clinical manifestations are more common in the elderly, especially primary osteoporosis in postmenopausal women [1]. With increase in aging population in China, the incidence of osteoporosis is on the rise, with serious impact on the psychology and life of the elderly. Therefore, the search for methods of prevention and treatment of osteoporosis has engaged the attention of medical researchers.

Studies have shown that estrogens reduce the differentiation of osteoblasts and osteoclasts, as well as the frequency of bone turnover. They also inhibit bone metabolism, and regulate osteoporosis. Long-term use of estrogen and progesterone in the treatment of osteoporosis leads to adverse side effects, thereby limiting their clinical application [2,3]. Quercetin is a phytoestrogen with estrogen antagonist property. It has anti-cancer, anti-depression, and anti-virus properties. In addition, quercetin induces apoptosis of osteoclasts [4]. In this study, the mechanism involved in anti-osteoporotic effect of quercetin was investigated in a rat model of osteoporosis.

EXPERIMENTAL

Experimental animals

Sixty SPF-grade, 3-month-old, female SD rats (mean weight = 240 ± 30 g) were obtained from Beijing Weitonglihua Co. Ltd. (animal license No.: SCXK, Beijing 2015-0003). The animals were maintained in SPF-class animal house with good ventilation and light, and were subjected to adaptation for 1 week at room temperature (20 - 23 ^{o}C) and relative humidity of 45 - 50 %, with *ad libitum* access to feed and drinking water. Thereafter, the rats were assigned to control, model and 3 quercetin groups [high-, middle- and low-dose given quercetin at doses of 200, 100 and 50 mg/kg/day, respectively), and diethylstilbestrol group (1.0 mg/kg/week), with 10 rats in each group. This research was approved by the Animal Ethical Committee of Hanchuan People's Hospital (approval no. 201961254), and was carried out in line with "Principles of Laboratory Animal Care" (NIH, 1985) [5].

Chemicals and reagents

The chemicals and reagents used, and their suppliers were quercetin (Chengdu Aikeda Chemical Reagent Co. Ltd., specification: 100g); ethylene estradiol tablets (Tianjin Lisheng Pharmaceutical Co. Ltd., specification: 0.5mg/tablet, Chinese Pharmaceutical Standard: H12020154); Bone Density Tester (LUNAR Company, DPX-L6843); ELISA Kit (Shanghai Biyuntian Biotechnology Co., Ltd.), and Automatic Biochemical Instrument (Thereto, USA).

Establishment of rat model of osteoporosis

The rats were clamped supinely and on aseptic operating table, and anesthetized with intraperitoneal injection of chloral hydrate (10%, 1ml/kg). After routine disinfection, their abdomens were cut open via incision, and the bilateral ovaries were excised, after which the incision site was disinfected and sutured. The control group was subjected to the same operation, but without ovary excision. Following the surgeries, the rats were fed for one week under suitable conditions.

Treatments

The high, medium and low dose quercetin groups received quercetin suspension at doses of 200, 100, 50 mg/kg/day, respectively. The diethylstilbestrol group was given diethylstilbestrol at doses of 1.0 mg/kg/week, while control rats and model rat groups received equivalent volumes of saline in place of quercetin or diethylstilbestrol. All treatments were given orally. After 6 weeks of continuous treatment, the rats were sacrificed prior to assay of the various biochemical indices.

Treatment indices

The physical activities and weights of the rats in each group were recorded. Prior to sacrifice, 5 mL of abdominal aortic blood was taken from each rat in the various groups, and the serum was separated and stored in refrigerator at -80^{o}C prior to use. Serum levels of BALP, E2 and BGP were assayed using ELISA.

The right femoral soft tissue was removed from each bone specimen, and BMD was measured with Dual Energy X-ray Absorptiometer (LUNAR Company, USA).

The AG-IX Biomechanics Universal Testing Machine was used to measure Maximum Load and Fracture Load. The left femur of each rat was placed on the test machine. The support span was 20 mm, and the loading speed was kept at 2 mm/min until the specimen was broken.

Statistical analysis

Measurement results are presented as mean ± standard deviation (SD). Groups were compared using single factor ANOVA, while χ^2 test was employed for analysis of counting data. All statistical analyses were done with SPSS version 20 software. Differences were taken as statistically significant at $p < 0.05$.

RESULTS

General profile of rats

As shown in Table 1, there were no significant abnormalities in mental state, diet, water

consumption, physical activity, stool texture and urine of rats in model and treatment groups, when compared with control group. Moreover, there were no significant differences in these parameters between the quercetin treatment groups and the model group. However, marked increases in weights of rats occurred in the model treatment rats, relative to control ($p < 0.05$). Rat weight was markedly lower in high-dose quercetin rats group than in model rats ($p < 0.05$). However, although rat weights in the middle and low-dose quercetin - diethylstilbestrol groups were lower than rat weight in the model group, the two groups were comparable, weight-wise ($p > 0.05$).

Table 1: Body weight of rats (n = 10)

Group	Dose	Body weight (g)
Control	0	173.21 ± 13.93
Model	0	264.10 ± 28.61*
Diethylstilbestrol	1.0mg/kg/week	249.45 ± 26.64*
High dose quercetin	200mg/kg/day	236.51 ± 26.03*#
Medium dose quercetin	100mg/kg/day	249.71 ± 21.77*
Low dose quercetin	50mg/kg/day	246.12 ± 22.18*

*$p < 0.05$, versus control; #$p < 0.05$, versus model

Changes in BALP, E2 and BGP levels in rats

As shown in Table 2, the results of serum test showed that BALP and BGP levels were markedly higher in model rats than normal control rats, while the levels of E2 were significantly lower. However, following treatment with quercetin, BALP and E2 levels were increased in middle- and high-dose groups, relative to model group, while BGP concentration in high-, medium- and low-dose quercetin rats were lesser than model group values ($p < 0.05$).

Changes in BMD level in rats

As shown in Table 3, there was lower BMD level in model group than in control group ($p < 0.05$). However, after treatment with quercetin at different concentrations, the BMD level in rats increased, and were significantly higher in quercetin treatment groups and diethylstilbestrol group than in model group ($p < 0.05$).

Bone biomechanical parameters in rats

The maximum load and breaking load were lower in model rats than in control rats ($p < 0.05$). However, quercetin and diethylstilbestrol administration led to significant improvement in these parameters. The maximum load and breaking load of the middle and high dose quercetin groups and diethylstilbestrol group were significantly higher than those of the model group ($p < 0.05$). These findings are shown in Table 4.

Table 2: Changes in BALP, E2 and BGP levels in rats (n = 10)

Group	BALP (U/L)	E_2 (ng/L)	BGP (ng/mL)
Control	97.61 ± 8.10	39.51 ± 3.56	2.56 ± 0.67
Model	113.20 ± 10.11#	12.51 ± 2.22#	5.76 ± 0.61#
Diethylstilbestrol	138.12 ± 15.14*	22.63 ± 2.31*	2.67 ± 1.12*
High dose quercetin	154.21 ± 17.13*	31.45 ± 3.02*	2.72 ± 1.06*
Medium dose quercetin	134.25 ± 12.01*	21.23 ± 1.91*	3.01 ± 1.16*
Low dose quercetin	115.20 ± 12.14	14.62 ± 2.34	3.57 ± 1.91*

*$p < 0.05$, versus model; #$p < 0.05$, versus control

Table 3: Changes in BMD levels of rats (n = 10)

Group	Dose	BMD
Control	0	0.144 ± 0.004
Model	0	0.117 ± 0.007#
Diethylstilbestrol	1.0mg/kg/week	0.146 ± 0.006*
High dose quercetin	200mg/kg/d	0.154 ± 0.003*
Medium dose quercetin	100mg/kg/d	0.134 ± 0.004*
Low dose quercetin	50mg/kg/d	0.119 ± 0.003*

*$p < 0.05$, versus model; #$p < 0.05$, versus control

Table 4: Changes in bone biomechanical parameters in rats in each group

Group	Maximum load	Breaking load
Control	142.16 ± 0.11	142.17 ±0.16
Model	86.27 ± 0.14#	86.23 ± 0.09#
Diethylstilbestrol	127.87 ± 0.29*	124.35 ± 0.24*
High dose quercetin	120.87 ± 2.39*	117.69 ± 1.78*
Medium dose quercetin	99.52 ± 6.08*	98.29 ± 1.68*
Low dose quercetin	94.28 ± 5.33	97.45 ± 1.53

*$p < 0.05$, versus control group; #$p < 0.05$, versus model

DISCUSSION

Osteoporosis is a metabolic disease which affects the elderly, especially postmenopausal women, with risks of neck fracture and vertebral compression fracture being much higher than

those in the premenopausal population. In severe cases, it can cause pneumonia and other complications [6]. Estrogen is secreted mainly by ovarian follicular cells, and it enhances bone density. Thus, estrogen deficiency is one of the main causes of female primary osteoporosis. At present, estrogen analogues, calcium, vitamin D and other anti-bone resorption and bone formation drugs are used in clinics for treating osteoporosis. However, prolonged use of these agents cause inflammatory reaction, breast cancer and other adverse reactions [7,8]. Quercetin is the most widely distributed polyhydroxy flavonoid in nature. Its toxicity and side effects are low, and it is does not readily produce drug resistance. Thus, quercetin has broad prospects for the treatment of osteoporosis [9].

The level of BALP, an osteoblast-specific enzyme in osteoblasts, directly reflects the activity of osteoblasts, and is a specific index of bone formation [10]. The secretion of parathyroid hormone increases during calcium deficiency, leading to the proliferation of osteoblasts and secretion of large amounts of BALP into the blood [11]. In this investigation, serum BALP levels of rats given different doses of quercetin for 6 weeks were markedly increased, relative to those in the model group. This suggests that quercetin affects the activity of osteoblasts at the early stage, and gradually promotes osteogenesis. Osteoclasts also secrete bone glaprotein (BGP), the serum levels of which reflect bone turnover. Moreover, BGP regulates normal mineralization of bone and inhibits cartilage mineralization. It has been used clinically as a reliable and specific index in for assessment of osteoblast activity and bone metabolism [12]. Studies have shown that postmenopausal osteoporosis results from estrogen deficiency which reduces the inhibition of osteoclasts, and increases bone resorption and bone formation [13].

In this study, the serum BGP rats treated with quercetin were decreased, when compared to model group. This indicates that quercetin mitigates osteoporosis by reducing the serum BGP content and bone turnover. In addition, the results of this study showed that the level of E2 increased significantly after quercetin treatment, suggesting that quercetin effectively enhanced the estrogen level of the patients. The mechanism involved in the quercetin-induced estrogen boost may involve the reduction of estrogen metabolites by inhibition of the expression of catechol-0-methyltransferase, and inhibition of bone resorption by interfering with the differentiation of osteoclasts [14].

Bone mineral density (BMD) is a sensitive index for predicting risk of fracture, and it is a parameter for accurate evaluation of bone mass *in vivo* [15]. In this study, the BMD of rats in quercetin treatment groups and diethylstilbestrol group increased significantly. This implies that quercetin can effectively reduce the incidence of fracture and increase BMD. Thus, it has a potential for use in the prevention and treatment of osteoporosis. Bone biomechanics refers to comprehensive response of bone mass, continuity of bone structure, cortical thickness and material properties of bone. It is used as an index for evaluating the effect of drugs on bone quality [16]. The results obtained in this study show that the maximum fracture load parameters in the quercetin- and diethylstilbestrol-treated rats were markedly higher than the corresponding values in model rats, suggesting that quercetin can prevent osteoporosis by improving the biomechanical properties of bone.

CONCLUSION

These results demonstrate that quercetin promotes bone formation, increases bone mineral density, reduces osteocalcin levels, and maintains bone biomechanics in ovariectomized rats, thereby preventing osteoporosis. Thus, quercetin has potentials as a drug for the treatment of osteoporosis in humans.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Fan Zhang conceived and designed the study, Hai Yang, Juntao Liu, Man Wang, Lu Wang, Lixiong Zhang and Fan Zhang collected and analysed the data, while Hai Yang wrote the manuscript.

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