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Effect of quercetin on estrogen receptor α and β expression in uterine tissues in ovariectomized rats

Lin Wang[1], Shumei Wang[1], Xiaofan Guo[2], Haijian Li, Yanna Lv[1*], Chunzhen Zhao[1*]

[1]Key Lab of Applied Pharmacology in Universities of Shandong, Weifang Medical University, Weifang, 261053, China

[2] Department of Pharmacy, Weifang Nursing Vocational College, Weifang, China

*Co-Corresponding author E-mail: chugzw@hotmail.com (Chunzhen Zhao); lynlyna@163.com (Yanna Lv)

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ABSTRACT:

Objective To explore the effect of quercetin on mRNA expressions of estrogen receptor α (ERα) and estrogen receptor β (ERβ) in ovariectomized (OVX) Sprague-Dawley (SD) rats. **Method** Thirty-two SD rats were randomly divided into 4 groups: sham group, ovariectomy group, quercetin group and estradiol group. The mRNA expression of ERα and ERβ in the uterus was determined by RT-PCR. The level of estradiol (E2) in the plasma was detected by ELISA. **Results** After ovariectomy, uterus coefficient, serum estradiol level and the mRNA expression of ERα and ERβ in the uterus were markly decreased in OVX rats. After treatment with quercetin, uterus coefficient, serum estradiol level and the mRNA expression of ERα and ERβ in the uterus were significantly increased. **Conclusion** Quercetin can improve menopausal syndrome in the OVX rats through regulating estrogen release and receptor expression.

Key words: ovariectomy; estrogen receptor, quercetin

INTRODUCTION:

Menopausal syndrome (MPS) occurs after female menopause because of ovarian dysfunction and hormonal metabolic disorder. Thus, postmenopausal women were often given to hormone-replacement therapy or estrogen-replacement therapy to improve their quality of life[1]. However, it has been induced concerns about the increase in the incidence of uterine carcinoma[2].

The mechanisms for its pathogenesis and progression are still unknown. It's well established that estrogen and its receptors play an important role in the occurrence of MPS[3, 4].

Lack of estrogen contributes that the wall of the uterus became thinned, epidermal cells reduced and endocrine dysfunction.

Quercetin, one of flavones in phytoestrogen, has been studied to found that it was closely related with estrogen correlative diseases, such as breast cancer[5], osteoporosis[6], and so on. Despite its estrogen-like activities, the effect of quercetin on estrogen receptor expression in the uterus is unknown. Estrogen has been identified that it can improve uterine and vagina atrophy after menopause[7].

Then, in this study we will investigate the treatment of quercetin on the level of hormones and mRNA expression of estrogen receptor-α and estrogen receptor-β. Consequently, investigation of possible protective effects of quercetin against MPS is executed.

Materials and methods

Animals

Thirty two female Sprague-Dawley rats weighing 200-230 g were housed in standard plastic cages, allowed free access to water and food, and maintained on a 12-12-hour light-dark cycle with light on at 7:00 am every day. All experiments were carried out in accordance with the National Institutes of Health Guide for the care and use of Laboratory animals, and approved by the Ethics Committee of Laboratory Animal Care and Welfare of Weifang Medical University.

Surgical preparation and ovariectomy, drug administration,

All experiments were conducted under general anaesthesia and paralysis[8].

Briefly, anaesthesia was induced by intraperitoneal injection of chloral hydrate (400 mg/kg).

The rats were bilaterally ovariectomized, while sham operation group were performed by exposing the ovaries only without isolation. We kept the animals in a box at 37 °C until they recovered from anesthesia. Each rat received intraperitoneal injections of penicillin G (80,000 U for each animal) for three consecutive days after surgery.

Two weeks after ovariectomy, drug administration was performed. The rats were randomly divided into four groups. Quercetin (Sigma Chemical Co., St. Louis, MO, USA) was diluted with sterile saline. 100 mg/kg of quercetin was administrated intragastrically. Estradiol group of conscious ovariectomized rats received a single subcutaneous injection of Estradiol (0.1 mg/kg, each) for four weeks. The control group received the same volume of intragastrical sterile saline.

Uterine coefficient calculation and sex hormones detection

The animals were sacrificed six weeks after ovariectomy. The ovaries were isolated with elimination of adipose tissues. Then weigh and calculate the uterine coefficient of all animals. The calculation formula is as follows:

$$Uterine\ coefficient = \frac{Wet\ weight\ of\ uterus}{Weight} \times 100\%$$

When sacrificed the animals, we firstly exposed the femoral artery and took blood for detection. Estradiol (E2) concentration in plasma was determined using ELISA kits (Neobioscience, China). All procedures were done according to the instructions of the manufactures.

Reverse transcription–polymerase chain reaction (RT-PCR)

Uterine tissues were dissected on ice and stored at -80 °C until use. Total RNA was extracted by using Trizol reagents (Invitrogen, USA). The primere sequences were designed using Primer Premier Software and the specificity of the oligonucleotide primers was verified using the program BLASTN. The primer sequences are as follows: ERα, forward 5’-AAT TCT GAC AAT CGA CGC CAG-3’ and reverse 5’-GTG CTT CAA CAT TCT CCC TCC TC-3’, ERβ, forward 5’-TTC CCG GCA GCA CCA GTA ACC-3’ and reverse 5’-TCC CTC TTT GCG TTT GGA CT-3’, β-actin, forward 5’-TAC AAC CTC CTT GCA GCT CC-3’ and reverse 5’-GGA TCT TCA TGA GGT AGT CAG TC-3’. PCR amplification was performed as follows: reaction mixtures were initially heated at 94 °C for 2 min, then at 94 °C for 60 s, 58 °C for 60 s, 72 °C for 60 s, for 35 cycles, and finally with a extension step of 72 °C for 10 min. The PCR products 10 µl were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

The density of each band was detected and analyzed with a UVP gel analysis system.

Statistical analysis

Data are expressed as mean ± SEM. The statistical analysis was done in SPSS 15.0. Significance of difference among groups was analyzed by a one-way analysis of variance (ANOVA) followed by Dennett's post hoc test. $P < 0.05$ was considered statistically significant.

Results

Effect of quercetin on the change of uterus weight

After ovariectomy, the uterus weight decreased obviously due to lack of sexual hormones from ovaries. Compared with sham operation group, ovariectomy significantly reduced uterine coefficient in ovariectomized rats of the model group. Quercetin administration (100 mg/kg) can significantly restored the reduction of uterine coefficient as comparing to ovariectomy group (Figure 1, $P < 0.05$). Estradiol treatment can also increased uterine coefficient compared with ovariectomy group ($P < 0.01$).

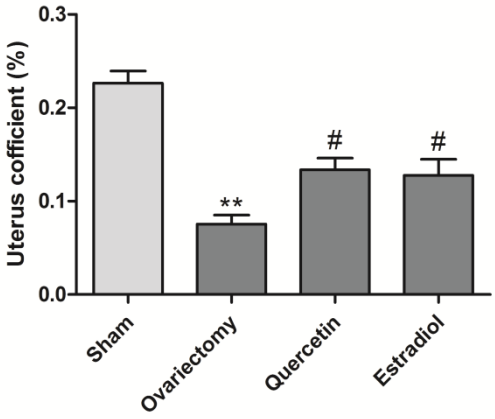

Fig.1: Uterus coefficient changes in all experimental groups

Effect of quercetin on the level of serum estrodiol (E2)

As shown in Figure 2, the level of serum estradiol in ovariectomy group, quercetin group, and estradiol group is markly decreased compared with sham operation control group ($P < 0.01$). There was a significant increase on the level of serum estradiol in quercetin group compared with model ovariectomy group ($P < 0.01$). And estradiol treatment also increased the level of serum estradiol (E2) compared with model ovariectomy group. Thus, quercetin and estradiol can increase the level of E2 in our study.

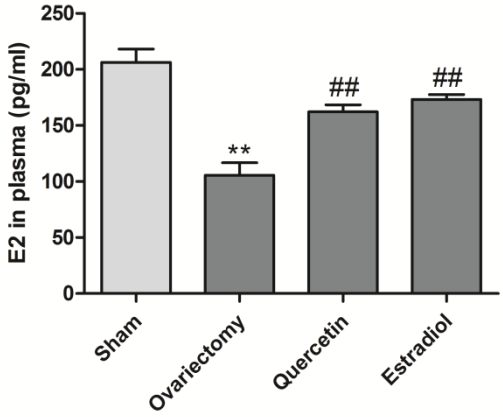

Fig.2: E2 level in plasma by ELISA in all experimental groups

Effect of quercetin on the expression of estrogen receptor α and β in the uterus

Results of real time PCR showed that ERα mRNA expression is significantly decreased in ovariectomy group compared with sham operation group (Figure 3A and B). ERβ mRNA expression also significantly decreased compared with control group as above described (Figure 3A and B). After quercetin treatment, both ERα and ERβ mRNA expression recovered obviously ($P < 0.05$, Figure 3C). Estradiol treatment can also increase the expression of ERα and ERβ mRNA expression.

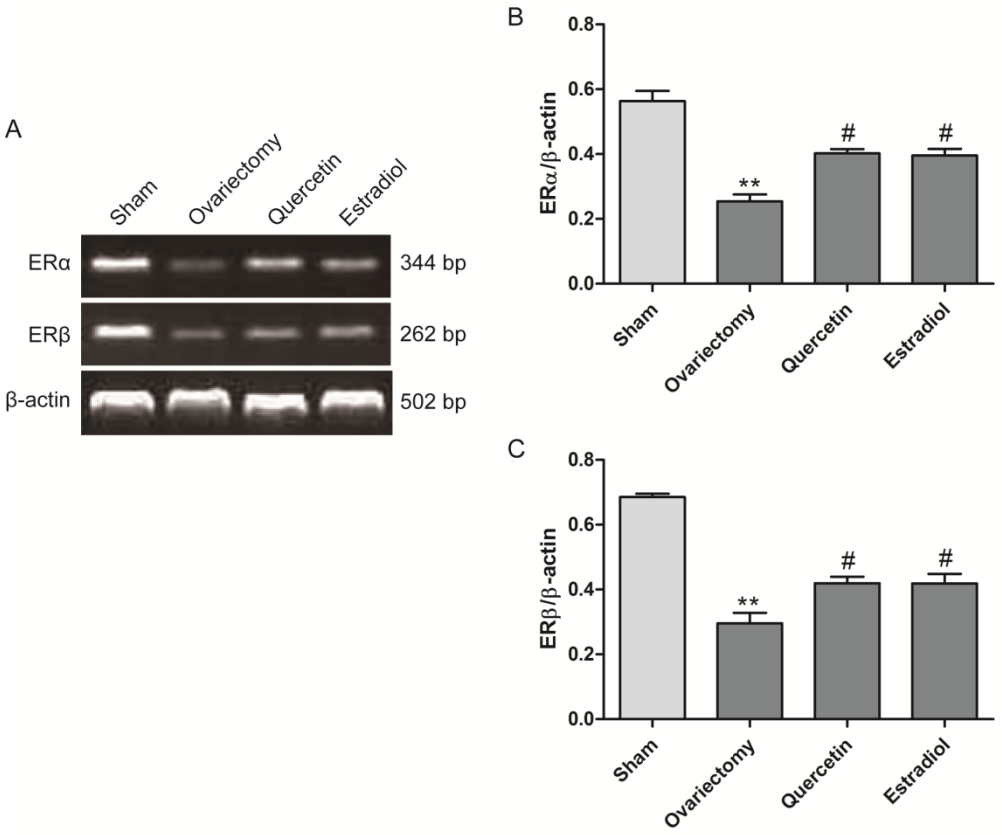

Fig.3: ERα and ERβ mRNA expression determined by RT-PCR

Discussion

There is a significant changement in the level of estrogen in the serum after menopause. Quercetin significantly recovered the weight and E2 level in the plasma by OVX, indicating that quercetin exerts estrogen-like effects. The recovery of uterine weight is based on endometrial proliferation and water absorption in the uterine endothelial membrane. It was reported that the vascular endothelial cell proliferation in vascularized endometrium is promoted by VEGF and also by E2[9]. Water absorption is mainly related with AQP2[10], a aquaporin subtype. Here we showed that quercetin increased the uterus coefficient in the uterus of OVX rats, suggesting that quercetin had estrogenic action. Lack of E2 stimulation, ovarian function declined in the postmenopausal women[11]. In the present study, treatment with 100 mg/kg of quercetin significantly increased the E2 level in the plasma.

Quercetin, [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one], is one of the most widely researched plant flavonoids[12]. Quercetin that is abundantly present in berries, apples and so on, play broad roles in anti-inflammation, antioxidation and immunomodulation[13]. It can directly bind to the estrogen receptor, categorized as a phytoestrogen. Like other flavonoid phytoestrogens, quercetin has a stronger binding affinity for receptor regulation. When the body is lack of estrogen, that is women in the menopause period of estrogen in the body at a relatively low level, phytoestrogen induces estrogen release. Our study found that 100 mg/kg of quercetin can increase the level of serum E2, which may be the effect of quercetin on the hypothalamic-pituitary-gonadal axis (HPGA). It was reported that quercetin upregulated the level of FSH and LH in the serum in regulation of the axis in the endometriosis[14].

Our results showed that quercetin can significantly upregulate the expression of ERα and ERβ in the uterine tissues. The uterus which is the main target organ of estrogen in women, change with the level of estrogen. Some plant estrogenic compounds such as Genistein have been reported to regulate morphological changes in the uterus[15]. The main function of uterus is regulated by changes of sexual steroid hormones, especially E2. In OVX rats, E2 level in the plasma was significantly decreased indicating the model is successfully built. ERα and ERβ mRNA expression in the uterine tissues were both reduced after ovariectomy. Estrogen receptor (ER) is a member of steroid receptor superfamily, which is a ligand-activated enhancer protein activated by the estrogen (17β-estadiol) and regulated via estrogen responsive elements[16]. The ER is encoded by two kinds of subtype genes, ERα and ERβ, functioned as a transcription factor modulating the expression of target genes[17]. Endogenous estrogen has a lower binding affinity to ERβ than ERα but both are transactivated by estrogen responsive element (ERE)[18].

Above results showed quercetin can regulate the expression of ERα and ERβ in the uterus, and then how quercetin regulates their expression and changes downstream pathways related with estrogen receptors is worthy of future study.

Conflicts of Interest

All the authors declare that there are no conflicts of interest.

Acknowledgement

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