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Original article

Glabridin and glycyrrhizic acid show no beneficial effect on the chemical composition and mechanical properties of bones in ovariectomized rats, when administered in moderate dose

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ABSTRACT

Background: One of the major causes of osteoporosis and bone fracture in postmenopausal women is estrogen deficiency. To prevent the fractures, and avoid the side effects of hormone replacement therapy, phytoestrogens including the isoflavonoids are used. In the presented study two constituents occurring in the licorice root—the isoflavane glabridin and triterpenoid saponin glycyrrhizic acid were examined on the skeletal system of ovariectomized rats.

Methods: The female Wistar rats were divided into five groups: control group, ovariectomized group as well as three ovariectomized groups treated with estradiol (0.2 mg/kg), glabridin (5 mg/kg) or glycyrrhizic acid (15 mg/kg). All substances were administered orally for 4 weeks. The estradiol served as a positive control. The mechanical properties of femoral diaphysis, tibial metaphysis and femoral neck were assessed using bending and compression tests. Moreover the chemical composition of the femur, tibia and L-4 vertebra – content of water, organic substances and minerals – was determined.

Results: Ovariectomy induced unfavorable changes in the skeletal system of the rats. Administration of glabridin and glycyrrhizic acid to the ovariectomized rats did not improve analyzed parameters of the bones.

Conclusion: Obtained results indicate, that the tested substances revealed no beneficial effect on the mechanical properties and chemical composition of the tested bones, thus they cannot be used as the osteoporosis protective agents.

Introduction

In postmenopausal women with deficiency of estrogens, the skeletal system degeneration and increased osteoporotic fracture risk is observed [1]. Total estradiol levels <5 pg/ml were associated with a 2.5-fold increase in hip and vertebral fractures in older women [2].

Due to increased prevalence of disabilities caused by bone fractures and high healthcare costs for fracture treatment in menopausal women, searching for methods to prevent fractures is crucial. The hormone replacement therapy (HRT) may be helpful in fracture prevention because it preserves and even increases bone mineral density at all skeletal sites, leading to a significant reduction in vertebral and non-vertebral fractures [2]. However, the principal complications of HRT are thromboembolic disease, stroke, cardiovascular disease, breast and endometrial cancer and gallbladder disease [3].

Selective estrogen receptor modulators (SERMs) are compounds that lack the steroid structure of estrogens, but interact with ERs as agonists (in bone) or antagonists in mammary glands and uterus (antiestrogenic activity) [4]. The SERM-like mechanism of action is exhibited by non-steroidal plant products—phytoestrogens. As natural SERMs they are good alternative for HRT—they exhibit beneficial effect on osteoporotic changes but do not increase the risk of breast and endometrial cancer development [5–7].

Glabridin, a substance occurring in licorice root can be classified as a phytoestrogen. The first scientific reports about its estrogen-like activity are from 2000 AD. Tamir et al. proved that glabridin binds to ERs in cells of human breast cancer. Glabridin at

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concentration above 15 μM caused antiproliferative activity associated with ERs stimulation [8]. It was also evidenced, that glabridin administered at doses of 2.5–250 μg to female rats stimulated creatine kinase activity (the marker of estrogenic activity) in the uterus, epiphyseal cartilage, femoral diaphysis, aorta and left ventricle. This effect was comparable to effect of estradiol administered at a dose of 5 μg. Other *in vitro* and *in vivo* studies, confirmed the agonistic activity of glabridin towards ERs [9,10].

There are also reports indicating that glabridin can bind to the ERs located in bone tissue [11]. Glabridin was applied at a concentration of 3000 nM into *in vitro* culture of human osteoblasts. It was also administered intraperitoneally at a dose of 3 μg to immature female rats. In both cases it was found that there was enhanced activity of creatine kinase in bones. Similar observations were described in ovariectomized rats, where animals were treated with glabridin at a dose of 100 μg.

In another study the agonistic properties of glabridin in endothelial cells and in aorta and heart of laboratory animals was confirmed [12]. In *in vitro* studies conducted by Simons et al. it has been proven, that glabridin in concentration of 6×10^{-6} M inhibits the response of cells on administration of the estradiol by 80%, what can indicate the antagonistic properties of this substance towards ERs [13].

Due to the agonistic and partial antagonistic activity towards ERs glabridin may be considered as natural SERM [10].

Except the glabridin, in the licorice root there is also a compound named glycyrrhizic acid with proved expectorant, anti-ulcer, anti-inflammatory and anti-allergic activities. There are also some reports indicating its beneficial effect on bone remodeling disorders [14,15].

The aim of the presented study was to investigate the effect of glabridin and glycyrrhizic acid on bone chemical composition and mechanical properties in rats with ovariectomy induced estrogen deficiency.

Materials and methods

The experiment was conducted on sexually mature female Wistar rats (initial body weight 217.2 ± 5.6 g). The study was approved by the Local Ethics Commission in Katowice (approval no. 70/2010). During the whole experiment the rats were fed with standard laboratory chow containing no soybean and had unlimited water supply. The animals were divided into 5 groups (n = 5):

C—control, non-ovariectomized rats

OVX—ovariectomized rats

ES—ovariectomized rats receiving estradiol (Estrofem, Novo Nordisk A/S, Bagsværd, Denmark) at a dose of 0.2 mg/kg *po*

GL—ovariectomized rats receiving glabridin (Sigma–Aldrich Co., St. Louis, Mo, USA) at a dose of 5 mg/kg *po*

GA—ovariectomized rats receiving glycyrrhizic acid (Sigma–Aldrich Co., St. Louis, Mo, USA) at a dose of 15 mg/kg *po*

Estradiol served as the positive controls in this experiment.

Analyzed substances were administered for 4 weeks once a day. Glabridin and glycyrrhizic acid were administered as suspended solids in distilled water at volume of 2 ml/kg. After drugs administration period, animals were sacrificed with the use of general anesthesia induced by mixture of ketamine and xylazine. Each of the rats had the left tibia and the left femur as well as the L-4 vertebra excised. After cessation, weight of the bones was recorded. Obtained bones were used in order to conduct the analysis of macrometric parameters, chemical composition and mechanical properties.

Assessment of chemical composition of bones

Evaluation of chemical components of bones included estimation of water, organic and mineral compounds. The assays were conducted on the femur, tibia and L-4 vertebra as previously described [16,17]. Briefly, the bones were lyophilized for 4 days (−53 °C, 0.03 mBa) and weighted. The difference between the weight after lyophilization and weight obtained after the isolation corresponded to the water content. Subsequently, the bones were mineralized for 48 h in muffle furnace in 640 °C, then weighted again. The difference in weight between lyophilized and mineralized bones referred to the organic substances content. The ash remained after the mineralization represented the weight of the mineral substances content.

Analysis of bone mechanical properties

The bone mechanical properties analysis of the femoral diaphysis, tibial proximal metaphysis and the femoral neck was performed as previously described [16–18]. For tibial diaphysis and tibial metaphysis the bending test was applied and following parameters were measured: maximal load, fracture load, displacement for maximal load, displacement for fracture load and Young's modulus. For femoral neck the compression test was performed and the maximal load was determined. These analyses were performed on Instron apparatus, model 3342 500 N connected to the computer with the Bluehill 2, version 2.14 software.

Statistical analysis

All results were evaluated by ANOVA test and presented as the means ± SEM.

Results

Effect of glabridin and glycyrrhizic acid on the body weight

After 4 weeks from the ovariectomy, the body weight gain was higher by 102% ($p < 0.001$) in the ovariectomized (OVX) rats, than in the non-ovariectomized control group (C). In the ovariectomized rats treated with estradiol (ES), the body weight gain was lower by 29.9% ($p < 0.05$), than in the OVX rats. After treatment with glabridin (GL) or glycyrrhizic acid (GA) no changes in body weight gain were recorded, when compared to the OVX rats (Table 1).

Effect of glabridin and glycyrrhizic acid on the bone weight

In the OVX rats, the weight of the femur was lower by 6.9% ($p < 0.05$) and the femur weight/body weight ratio was lower by 13.5% ($p < 0.001$) than in the C rats. In the ES rats only the femur weight/body weight ratio was higher by 6.4% ($p < 0.01$) when compared to the OVX rats. In the remained groups there were no significant changes in the femoral weight or the femur weight/body weight ratio in comparison to the OVX group. The decrease of the tibia weight by 6.2% and the tibia weight/body weight ratio by 12.5% ($p < 0.01$) was recorded in the OVX rats when confronted with the C rats. In the ES, GL and GA rats, there were no significant changes in the tibia weight, when compared to the OVX rats. In the ES rats the tibia weight/body weight ratio was higher by 9.3% than in the OVX group. In the OVX rats, the weight of the L-4 vertebra and the L-4 vertebra/body weight ratio were lower by 12.7% and 19.2% ($p < 0.05$) respectively, than in the C group. In the ES group, the increase of the L-4 vertebra weight/body weight ratio by 15.3% was recorded in comparison to the OVX group. Administration of glabridin or glycyrrhizic acid to the ovariectomized rats did not