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EXPERIMENTAL STUDY

Puerarin affects bone biomarkers in the serum of rats with intrauterine growth restriction

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Abstract

OBJECTIVE: To investigate serum bone biomarkers in rats with intrauterine growth restriction (IUGR) in order to determine the effects of puerarin on bone metabolism.

METHODS: A rat model of IUGR was induced using a low protein diet during pregnancy. The offspring were given puerarin or an identical volume of saline via subcutaneous abdominal injection. All rats were studied at 1, 3, and 8 weeks of age. Serum biomarkers of bone formation, including insulin-like growth factor-1 (IGF-1), bone-specific alkaline phosphatase (BALP), osteocalcin (OC), osteoprotegerin (OPG), receptor-activator of nuclear factor-κB ligand (RANKL), as well as blood levels of calcium and phosphorus were measured.

RESULTS: Serum BALP, OPG, IGF-1, and OC levels, as well as the OPG/RANKL ratio, were lower in the IUGR group compared with the control group at 1 week of age (P = 0.024, 0.011, 0.014, 0.004, and 0.002, respectively). At 3 weeks of age, the serum BALP and OC levels were higher in the protein-restricted group compared with the control group (P = 0.003 and 0.001, respectively). A comparison between the IUGR plus puerarin intervention group and the IUGR group revealed differences in the levels of BALP and IGF-1 at 3 weeks of age (P = 0.008 and 0.003, respectively). In addition, serum OPG and OC levels and the OPG/RANKL ratio were higher at 8 weeks of age (P = 0.044, 0.007, and 0.016, respectively). No differences in serum calcium and phosphorus levels were observed among the three groups.

CONCLUSION: Our study demonstrates that the bone microenvironment of the fetus can be altered by a low protein maternal diet and that puerarin can reverse these effects. These results indicate that the nutritional environment plays an important role in early skeletal development and that the bone turnover of IUGR rats can be altered by puerarin treatment.

Key words: Puerarin; Fetal growth retardation; Osteogenesis; Bone biomarker

INTRODUCTION

In humans, the development of the fetus can have major influences on the adult phenotype. A good example of this phenomenon is seen in intrauterine growth restriction (IUGR), which can be defined as when the estimated fetal weight (EFW) is below the 10th percentile of healthy fetus of the same gestational age. Causes of IUGR include malnutrition, placental insufficiency, and congenital anomalies. IUGR affects approximately 10%-15% of pregnant women.[1] Fetuses with IUGR

have a higher risk of perinatal morbidity, mortality and long-term sequelae, including osteoporosis, obesity, insulin resistance, hypertension, and brain injury.

Bone formation requires adequate supplies of energy, amino acids, minerals, and vitamins. Some studies have indicated that the intrauterine environment plays an important role in skeletal development.[2] In recent years, many studies have reported that protein deficiencies during pregnancy have an adverse effect on bone growth. Mehta *et al* [3] reported that maternal protein restriction during pregnancy results in reduced bone area and bone mineral content (BMC). Lanham *et al* [4] also demonstrated that a low protein maternal diet affects the bone structure of female offspring when they reach an elderly age using micro-computed tomography (micro-CT) and mechanical testing. Thus, bone growth may have been affected by IUGR, which was induced by protein deficiency during pregnancy.

In recent years, estrogen replacement therapy has been considered an effective approach for preventing age-related bone loss in females.[5] Puerarin [4H-1-benzopyran-4-one,8-b-D-glucopyranosyl-7-hydroxy-3-(4-hydroxy-phenyl)] is a major phytoestrogen, isolated from Gegen (*Radix Puerariae Lobatae*), which binds to estrogen receptors and has weak estrogenic activity. Wang *et al*.[6] demonstrated that puerarin reduces the loss of bone density that occurs in ovariectomized mice. Wong *et al* [7] also demonstrated that puerarin induces local increases in new bone formation in a collagen matrix.

This study was based on the hypothesis that serum bone biomarkers may differ between rats with IUGR and healthy controls. We further hypothesized that puerarin treatment could promote recovery of serum bone biomarkers to normal levels. Serum levels of insulin-like growth factor-1 (IGF-1), osteocalcin (OC), bone specific alkaline phosphatase (BALP), osteoprotegerin (OPG), and receptor-activator of nuclear factor kappa beta ligand (RANKL) were measured to determine the association between these biomarkers and IUGR. In addition, circulating levels of calcium and phosphorus were evaluated.

MATERIALS AND METHODS

Animals and treatments

All experimental designs and procedures received approval by the Animal Ethics Committee of Central South University (Changsha, China). Specific pathogen free -grade Sprague-Dawley rats [18 females, 9 males; 3 months of age, weighing (220 ± 20) g], purchased from the Experimental Animal Center of Central South University [certificate of quality No. SCXK (xiang) 2009-0012], were housed individually at 25 ℃, with a 12∶12 h light-dark cycle. Successful mating was confirmed after observing sperm in a vaginal smear; the day of successful mating day was used as day zero of gestation. After gestational day 0, pregnant dams were housed individually and were randomly allocated to either a normal protein diet containing 21% protein (w/w), for the control group, or a low protein diet containing 10% protein (w/w) for both the IUGR and the IUGR plus puerarin intervention groups; both diets had the same caloric value.[4,8] Offspring of both the IUGR and IUGR plus puerarin intervention groups were randomly divided into two subgroups using a random number table. One group received 50 $mg \cdot kg^{-1} \cdot d^{-1}$ of puerarin (Tianjin Pharmaceutical Group Co., Xinzheng Ltd., Xinzheng, Henan, China) via subcutaneous abdominal injection for 1 week, whereas the other group received an injection of the same volume of saline. Experimental samples were harvested after 1, 3, and 8 weeks. Blood was collected in pyrogen-free tubes after centrifugation. The supernatants from the serum samples were frozen at − 80 °C until the time of the assay.

Plasma OPG levels were measured using an enzyme-linked immunosorbent assay (ELISA; SEA108Ra, Cloud-clone Co., Houston, TX, USA) with a detection range of 0.156-10 ng/mL and a sensitivity of 0.053 ng/mL. Plasma IGF-1 levels were also measured using an ELISA (SEA050Ra, Cloud-clone Co., Houston, TX, USA) with a detection range of 78.125-5000 pg/mL and a sensitivity of 30 pg/ml. Plasma osteocalcin levels were measured using an ELISA (SEA 471Ra, Cloud-clone Co., Houston, TX, USA) with a detection range of 15.625-1000 pg/mL and a sensitivity of 6.3 ng/mL. Plasma BALP levels were measured using an ELISA (SEB 091Ra, Cloud-clone Co., Houston, TX, USA) with a detection range of 0.781-50 ng/mL and a sensitivity of 0.28 ng/ml. Plasma RANKL levels were measured using an ELISA (SEA855Ra, Cloud-clone Co., Houston, TX, USA) with a detection range of 15.625-1000 ng/mL and a sensitivity of 5.3 ng/mL. Serum calcium and phosphorus levels were measured using the ARCHITECT c8000 system (Abbott Co., Chicago, IL, USA).

Statistical analysis

The data are represented as the mean ± standard deviation ($\bar{x} \pm s$). Multiple samples were compared using analysis of variance. Several sets of multiple samples were analyzed *via* several pairwise comparisons using the analysis of variance least significant difference method. Sample rates were compared using the χ^2 test. All computations were performed using SPSS 17.0 software (SPSS Inc., St. Louis, MO, USA). Differences with $P < 0.05$ were considered statistically significant.

RESULTS

The birth weights of offspring whose mothers were fed a restricted protein diet (i.e., the IUGR and IUGR plus puerarin intervention groups) met the criteria for IUGR and were significantly lower than those of the control group ($P > 0.001$). After 1 week, the body