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Original Research Article

Effect of *Dihuang rougui* decoction on ovariectomy-induced osteoporosis in rats

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Abstract

Purpose: *To investigate the effect of Dihuang Rougui Decoction (DRD) on ovariectomy-induced osteoporosis in rats.*
Methods: *Female Sprague-Dawley rats were randomly assigned to a normal group (control) and five ovariectomy (OVX) subgroups: OVX with vehicle (OVX), OVX with positive control drug (alendronate sodium tablets, 1.6 mg/kg/week), and OVX + DRD (75, 150 or 300 mg/kg/day). After the rats were subjected to ovariectomy for 4 weeks, fosamax or DRD were administered daily (orally) for 16 weeks. The bone mineral density (BMD) of the L4 vertebrae and right femurs of the rats was evaluated. Serum hormones estradiol (E2), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels and serum alkaline phosphatase (ALP), osteocalcin (OC) and telopeptides of collagen type I (CTx) levels of the rats were determined. The bone tissue morphology of the rats was examined by microscopy.*
Results: *The results show that DRD dose-dependently inhibited bone mineral density (BMD) reduction of L4 vertebrae and femurs (both $p < 0.05$). DRD significantly increased serum E2, FSH and LH levels ($p < 0.05$) in the osteoporotic rats, and significantly lowered serum ALP, CTx and OC concentrations, compared to OVX group ($p < 0.01$). Compared with OVX model group, bone trabeculae in all three DRD groups and nilestriol groups were wider, and the space and connections markedly increased. Furthermore, the medullary cavity reduced in size.*
Conclusion: *These findings indicate that DRD mitigates OVX-induced osteoporosis in rats, and thus, the decoction has a potential for clinical management of osteoporosis patients.*

Keywords: *Dihuang Rougui decoction, Osteoporosis, Ovariectomy, Bone mineral density, Serum bone marker, Bone tissue morphology*

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INTRODUCTION

Postmenopausal osteoporosis is a type of systemic bone disease characterized by a reduction in bone density, degradation of bone microstructure and an alteration in serum markers of bone metabolism, such as alkaline phosphatase (ALP), estradiol (E2) and interleukin-6 (IL-6) in postmenopausal females. This results in bone fragility and an increased risk of fracture. Approximately one third of postmenopausal females suffer from

osteoporosis and this is usually due to the marked reduction in estrogen levels [1].

According to data released by World Health Organization (WHO), osteoporosis affects approximately million people throughout Europe, USA, and Japan [2]. Incidence of osteoporosis increases dramatically with life expectancy. Accordingly, the risk of osteoporotic fractures and their associated costs is rising rapidly due to aging population [3]. In the elderly, hip fractures are closely associated with mortality [4]. Hormone deficiency is known to impair cancellous metaphyseal and reduce BMD in humans and animals. Therefore, estrogen deficiency in post-menopausal women has been regarded as a critical cause of this population's susceptibility to osteoporosis [5]. Osteoporosis is twice as common in women as in men [6], and approximately one in three women over 50 years old experiences an osteoporotic fracture in her life time [7].

Clinically, hormone replacement therapy (HRT) has been a popular therapeutic strategy designed for postmenopausal osteoporosis [8,9]. However, long-term application of HRT has potential malignant effects on reproductive tissues [10-12]. Other medicines that stimulate bone formation (e.g., growth hormone, sodium uoride, and parathyroid hormone) or inhibit bone resorption (e.g., bisphosphonates and calcitonin) may prevent bone loss progression in established osteoporosis. However, these drugs are not effective for a large proportion of the world population, especially in developing countries, and their drugs have side effects, such as gastrointestinal reactions, cancers, osteonecrosis of the jaw, and reduced skeletal strength [13,14]. Consequently, to substitute or reduce the medicines used currently, there are efforts to develop new drugs with improved therapeutic efficacy, fewer undesirable side effects, and lower cost.

Dihuang Rougui Decoction is a traditional Chinese medicine composed of *Codonopsis pilosula (Franch.) Nannf.* 3 gram, *Rehmannia glutinosa (Gaetn.) Libosch. ex Fisch. et Mey.* 4 gram, *Pinellia ternata (Thunb.) Breit.* 2 gram, *Angelica sinensis (Oliv.) Diels* 2 gram, *Evodia rutaecarpa (Juss.) Benth.* 7 gram and *Cinnamomum cassia Presl* 6 gram, which has been commonly used as an anti-aging agent, anti-osteoporosis and aphrodisiac in Chinese traditional medicine [15].

This study was carried out to investigate the therapeutic effects of DRD on ovariectomy-induced osteoporosis in rats.

EXPERIMENTAL

Preparation of *Dihuang Rougui* decoction

The herbal samples of *Dihuang Rougui* Decoction were collected from Guilin City, Guangxi Province in China in November 2016. Taxonomic identification of the plant was performed by Professor Gang Hu of College of pharmacy, Liaoning University of Traditional Chinese Medicine, in Shenyang of China. A voucher specimen (no. DRD 201611023) was deposited in the herbarium of college of pharmacy, Liaoning University of Traditional Chinese Medicine, Shenyang, China for future reference. *Dihuang Rougui* Decoction was provided by the manufacturing laboratory of Orthopedic Hospital of Shenyang.

Animals and treatments

Healthy six-month-old female Sprague-Dawley rats (weighing 220 ± 20 g) were provided by Liaoning Province Experimental Animal center (certificate no. SYXK2002-0003). The animals had free access to feed and water, and were allowed to acclimatize for at least one week before use. The rat experiment was approved by Animal Care and Use Committee of Orthopedic Hospital of Shenyang (approval ref no. 200607856) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [16].

Sixty rats were randomly divided into six groups of ten a group (control) and five ovariectomy (OVX) subgroups, that is, OVX with vehicle (OVX), OVX with positive control drug, alendronate sodium tablets (ASD, 1.6 mg/kg/week), and OVX with DRD doses 75, 150 or 300 mg/kg/day.

BMD measurement

The BMD of the L1-5 vertebrae and right femurs was estimated using dual-energy X-ray absorptiometry scanning (DEXA, GE Healthcare, USA) with small animal measurement. The measurement was expressed as mineral grams per cm^2 of surface area. Scans were performed by the same blinded technician.

Serum E2, FSH and LH levels in rats treated with DRD

After the rats were sacrificed by cervical dislocation, serum was obtained by removing the eyeball. And serum E2, FSH and LH levels of rats were determined by ELISA method (provided by Nanjing built Biological

Engineering Research Institute Co., Ltd. Nanjing, China).

Serum bone marker in rats treated with DRD

Rat serum alkaline phosphatase (ALP), osteocalcin (OC) and telopeptides of collagen type I (CTx) levels were determined by ELISA method (provided by Nanjing built Biological Engineering Research Institute Co., Ltd. Nanjing, China).

Statistical analysis

The data are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA combined with Bonferroni's multiple comparison test using SPSS 16.0. Differences were considered statistically significant at $p < 0.05$.

RESULTS

BMD of L1-5 vertebrae and femurs

The BMD of L1-5 vertebrae and femurs is presented in Table 1. These results demonstrate that OVX significantly decreased BMD in the L4 vertebrae and femurs compared to the control group ($p < 0.05$). Compared to the OVX group, DRD treatment significantly decreased the BMD in OVX-induced L4 vertebrae and femurs (all $p < 0.05$) in a dose-dependent manner. The positive control drug Fosamax also significantly increased the BMD of the L4 vertebrae and femurs (both $p < 0.05$), which was similar to that observed in the H-DRD group ($p > 0.05$).

Table 1: Effect of DRD on BMD of L4 vertebrae and femurs (n = 10)

Group	Dose (mg/kg)	BMD of vertebrum (g/cm^2)	BMD of femur (g/cm^2)
Control	-	0.92±0.04*	0.64±0.05*
OVX	-	0.25±0.05	0.16±0.04
ASD	1.6	0.32±0.04*	0.34±0.03*
L-DRD	75	0.30±0.04*	0.25±0.04*
M-DRD	150	0.33±0.03*	0.23±0.04*
H-DRD	300	0.72±0.04*	0.44±0.03*

*$P < 0.05$ and **$p < 0.01$ versus OVX group. L-DRD = low dose of DRD, M-DRD = middle dose of DRD, H-DRD = high dose of DRD, ASD: Alendronate Sodium Tablets

Effect of DRD on serum E2, FSH and LH levels

Compared to control group, serum E2, FSH and LH levels decreased significantly in OVX group rats ($p < 0.01$). Compared to the OVX group, DRD treatment significantly increased serum E2, FSH and LH levels ($p < 0.05$) in a dose-dependent manner in rats (Table 2).

Table 2: Effect of DRD on serum hormone levels (n = 10)

Group	Dose (mg/kg)	E2 (pmol/L)	FSH (IU/L)	LH (mIU/mL)
Control	-	8.57±0.18**	2.84±0.05**	3.43±0.12*
OVX	-	1.37±0.13	0.41±0.03	1.24±0.06
ASD	1.6	4.26±0.15**	1.78±0.04*	2.26±0.04*
L-DRD	75	2.46±0.22	0.92±0.04	1.82±0.11
M-DRD	150	3.65±0.28*	1.51±0.05*	2.38±0.06*
H-DRD	300	4.34±0.19**	1.80±0.03**	2.82±0.07*

*$p < 0.05$ and **$p < 0.01$ versus OVX group. L-DRD: low dose of DRD, M-DRD: middle dose of DRD, H-DRD: high dose of DRD, ASD: Alendronate Sodium Tablets

Effect of DRD on serum ALP, CTx and OC concentrations

Serum ALP, CTx, and OC concentrations in the OVX group were significantly higher as compared to the control group. After four weeks of treatment, the DRD 300 mg/kg-treated group showed significantly lower serum ALP, CTx and OC concentrations compared to the OVX group ($p < 0.01$) (Table 3).

Table 3: Effect of DRD on serum ALP, CTx, and OC levels (n = 10)

Group	Dose (mg/kg)	ALP (U/L)	CTX (ng/mL)	OC (ng/mL)
Control	-	123.5±3.8	27.6±1.7	46.4±2.0
OVX	-	256.5±5.8**	82.1±1.5**	82.3±2.4**
ASD	1.6	177.2±4.9**	53.3±2.4**	51.3±1.3**
L-DRD	75	223.6±5.8	76.1±2.3	78.5±1.8
M-DRD	150	176.5±4.3*	67.8±1.4*	73.2±1.3*
H-DRD	300	152.4±3.9**	52.3±2.3**	60.5±1.4**

*$P < 0.05$ and **$p < 0.01$ versus OVX group. L-DRD: low dose of DRD, M-DRD: middle dose of DRD, H-DRD: high dose of DRD, ASD: alendronate sodium tablets

DISCUSSION

Postmenopausal osteoporosis is a systematic imbalance in which the speed of bone resorption is greater than bone formation. This disease is caused by estrogen deficiency and results in micro-architectural changes, particularly bone remodeling. Certain critical molecules co-ordinate the actions of osteoblasts and osteoclasts during bone remodeling. Therefore, the development of new preventive and therapeutic drugs for osteoporosis is urgently needed. In recent decades, Chinese medicinal herbal extracts have been extensively investigated for their pharmacological effects related to bone protection [17]. Decreased BMD

is one of the major factors that jeopardize bone strength, resulting in increased susceptibility to fractures. Thus, BMD measurement can best predict fracture risk [18]. The results in the present study showed that OVX reduced BMD in the right femurs and L4 vertebrae, which are rich in trabecular bone, while treatment with DRD dose-dependently prevented the decreases in BMD. Although BMD is among the strongest predictors of facture resistance, both empirical observations and theoretical analyses show that the biomechanical properties of bone and trabecular microarchitecture influence trabecular bone strength as well [19].

Estradiol plays an important role in sclerotin of humans. When female serum estradiol level decreases significantly, osteoporosis will occur [20]. Compared to the OVX group, DRD treatment significantly increased serum E2, FSH and LH levels in a dose-dependent manner in rats. In our experiments, OVX resulted in significant decrease in femur BMD after four weeks. The BMD loss was accompanied by a significant increase in bone remodeling, as evidenced by the increased biochemical bone turnover markers, such as serum ALP, CTx, and OC levels [21]. In the present study, oral administration of DRD at the dose 248 mg/kg significantly decreased BMD loss, which was accompanied by the decrease in serum ALP, CTx, and OC levels compared to a OVX control group. These results suggest that Bushen Jiangu Decoction could decreases bone loss by inhibiting bone turnover induced by OVX.

CONCLUSION

The findings of this work indicate that DRD prevents OVX-induced osteoporosis in rats, and therefore can potentially be used for the treatment of postmenopausal osteoporosis of elderly women in future. However, further studies on the toxicity and safety of the decoction, as well as its activity in humans are required.

DECLARATIONS

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Conflict of interest

The authors declare that there is no any conflict of interest with regard to this study.

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. Ye Liu designed all the experiment and revised the paper. Ming-chang Du, Bo Wu, Xu Ma, Liang-quan Zhai, Xun Fu performed the experiment and Zheng-bo Yang wrote the paper. Ming-chang Du, Bo Wu and Xu Ma contributed equally to this work, and they are co-first author.

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