Original Research Article

Anti-osteoporotic effect of Gengnian Jianshen decoction in rats

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Abstract

Purpose: To investigate the therapeutic effect and mechanism of action of Gengnian Jianshen Decoction (GJD) on ovariectomy-induced osteoporosis in rats.
Methods: Female Sprague-Dawley rats were randomly assigned to a sham-operated group (control), and five ovariectomy (OVX) sub-groups, that is, OVX with vehicle (OVX), OVX with Xianling Gubao capsule (positive control drug, 120 mg/kg/day), and OVX with GJD doses (70, 140, and 280 mg/kg/day). The treatments were given orally daily for 16 weeks (starting 4 weeks after the rats were subjected to ovariectomy. Bone mineral density (BMD) of the L4 vertebrae and right femur of each rat was estimated. Serum levels of estradiol (E2), follicle-stimulating hormone (FSH) and luteinizing hormone (LH), as well as interleukin-6 (IL-6) and insulin-like growth factor 1(IGF-1) of rats in all the groups were determined using ELISA.
Results: The results showed that GJD dose-dependently inhibited BMD reduction in L4 vertebrae and femur, and significantly increased serum E2, FSH and LH levels ($p < 0.05$) in the osteoporotic rats. Moreover, GJD significantly decreased serum IL-6 levels and increased levels of IGF-1 ($p < 0.05$).
Conclusion: These findings indicate that GJD prevents OVX-induced osteoporosis in rats without hyperplastic effects on the uterus. Thus, GJD has potential for use in the treatment of post-menopausal osteoporosis.

Keywords: Gengnian Jianshen decoction, Osteoporosis, Ovariectomy, Bone mineral density

INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by reduced bone mass and microarchitectural deterioration of bone tissue with concomitant increase in bone fragility and susceptibility to fractures [1]. According to data released by the World Health Organization (WHO), osteoporosis affects several million people throughout Europe, USA, and Japan [2]. The incidence of osteoporosis increases dramatically with life expectancy. Accordingly, the risk of osteoporotic fractures and their associated costs are rising rapidly due to increases in aging populations [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 factor in the susceptibility of this population 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 for post-menopausal osteoporosis [8, 9]. However, long-term application of HRT has potential malignant effects on reproductive tissues [10-13]. Other medicines that stimulate bone formation (e.g., growth hormone, sodium fluoride, and parathyroid hormone), or inhibit bone resorption (e.g., bisphosphonates and calcitonin) may prevent progression of bone loss in established osteoporosis. However, these drugs are not effective for a large proportion of the world population, especially in developing countries. In addition, they have side effects such as gastrointestinal reactions, cancers, osteonecrosis of the jaw, and reduced skeletal strength [14,15]. Consequently, there are efforts to develop new drugs with improved therapeutic efficacies, fewer undesirable side effects, and lower costs, so as to substitute or reduce the usage of medicines currently in use.

Gengnian Jianshen Decoction (GJD) is an empirical formula that has been used for the treatment of osteoporosis in China for many years. This study was aimed at investigating the therapeutic effects and mechanism of action of GJD on ovariectomy-induced osteoporosis in rats.

EXPERIMENTAL

Preparation of *Gengnian Jianshen* decoction

Gengnian Jianshen decoction was composed of *Uncaria rhynchophylla（Miq.）Miq. ex Hav.* (15g)*, Poria cocos (Schw.) Wolf* (10g)*, Pseudostellaria heterophylla (Miq.) Pax ex Pax et Hoffm.* (15g)*, Rehmannia glutinosa* (10g)*, Dioscorea opposita Thunb.* (10g)*, Cornus officinalis Sieb. et Zucc.* (10g)*, Psoralea corylifolia Linn.* (15g)*, Dipsacales* (10g)*, Achyranthes bidentata Blume.* (10g)*, Eucommia ulmoides* (10g)*, Triticum aestivum L* (30g)*, Apatite* (20g) *and Nelumbo nucifera Gaertn.* (5g)*.* These herbs were mixed, and decocted with 1700 mL of water for 45 min. The decoction was performed twice to maximize GJD extract yield. The extract was concentrated and filtered through a membrane filter to obtain GJD with a concentration of 1200 µg/mL.

Animals and treatments

Healthy, six-month-old female Sprague-Dawley rats (weighing 220 ± 20 g) were provided by Jiangsu Animal center (certificate no. SYXK 2003-0007). 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 Second Affiliated Hospital of Nanjing University of Chinese Medicine (approval ref no. 20100308), and was carried out in compliance with the Directive 2010/63/EU on the handling of animals used for scientific purposes [16].

Sixty rats were randomly divided into six groups of ten rats: a sham-operated group (control) and five ovariectomy (OVX) sub-groups, that is, OVX with vehicle (OVX), OVX with positive control drug (Xianling Gubao capsule, 120 mg/kg/day), and OVX with GJD doses (70, 140 and 280 mg/kg/day).

BMD measurement

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

Determination of serum E2, FSH, LH, IL-6 and IGF-1 levels

After the rats were sacrificed by cervical dislocation, and serum levels of E2, FSH and LH, and IL-6 and IGF-1 levels were determined by ELISA.

Statistical analysis

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

RESULTS

BMD of L1-5 vertebrae and femurs

Values of BMD of the L1-5 vertebrae and femurs are presented in Table 1. These results demonstrate that OVX significantly decreased BMD in the L4 vertebrae and femurs, when compared with the control group ($p < 0.05$). Compared to the OVX group, GJD treatment significantly and dose-dependently inhibited decreases in BMD in OVX-induced L4 vertebrae and femurs ($p < 0.05$). The positive control drug *Xianling Gubao* capsule also significantly increased the BMD of the L4 vertebrae and femurs ($p < 0.05$), in a manner similar to that observed in the H-GJD group ($p > 0.05$).

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

Group	Dose (mg/kg)	BMD of vertebra (g/cm^2)	BMD of femur (g/cm^2)
Control	-	0.61 ± 0.07*	0.38 ± 0.04*
OVX	-	0.23 ± 0.04	0.13 ± 0.04
XGC	120	0.35 ± 0.05*	0.26 ± 0.04*
L-EBME	70	0.26 ± 0.03*	0.16 ± 0.04*
M-EBME	140	0.38 ± 0.04*	0.25 ± 0.04*
H-EBME	280	0.58 ± 0.03*	0.34 ± 0.03*

*$P < 0.05$ and **$p < 0.01$ versus OVX group. XGC: *Xianling Gubao capsule*, L-EBME: low dose of GJD, M-EBME: middle dose of GJD, H-EBME: high dose of GJD

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

Group	Dose (mg/kg)	E2 (pmol/L)	FSH (IU/L)	LH (mIU/mL)
Control	-	5.68 ± 0.21**	1.96 ± 0.07**	2.88 ± 0.12*
OVX	-	1.45 ± 0.16	0.54 ± 0.06	1.24 ± 0.08
XGC	120	3.96 ± 0.25**	1.36 ± 0.06*	2.34 ± 0.05*
L-EBME	70	2.36 ± 0.37*	0.87 ± 0.05	1.78 ± 0.14
M-EBME	140	3.26 ± 0.35*	1.24 ± 0.04*	2.32 ± 0.08*
H-EBME	280	4.89± 0.14**	1.56 ± 0.04**	2.67 ± 0.06*

*$P < 0.05$ and **$p < 0.01$ versus OVX group. XGC: *Xianling Gubao* capsule, L-EBME: low dose of GJD, M-EBME: middle dose of GJD, H-EBME: high dose of GJD

Table 3: Effect of GJD on serum IL-6 and IGF-1 levels (n = 10)

Group	Dose (mg/kg)	IL-6 (pmol/L)	IGF-1 (pmol/L)
Control	-	27.25 ± 2.21	14.33 ± 1.18
OVX	-	49.33 ± 3.46*	3.42 ± 0.94**
XGC	120	32.43 ± 2.87*	7.59 ± 1.24*
L-EBME	70	46.37 ± 3.12	4.93 ± 1.16
M-EBME	140	40.42 ± 2.36*	7.13 ± 1.35*
H-EBME	280	27.36 ± 2.57*	9.25 ± 1.04**

*$P < 0.05$ and **$p < 0.01$ versus OVX group. XGC: *Xianling Gubao* capsule, L-EBME: low dose of GJD, M-EBME: middle dose of GJD, H-EBME: high dose of GJD

Effect of GJD on serum levels of E2, FSH and LH

Serum E2, FSH and LH levels decreased significantly in OVX group rats relative to the control group ($p < 0.01$). Compared to the OVX group, GJD treatment significantly and dose-dependently increased serum E2, FSH and LH levels ($p < 0.05$) in the osteoporotic rats.

Effect of GJD on serum IL-6 and IGF-1 levels

Serum IL-6 level increased and IGF-1 level decreased significantly in OVX group rats, relative to the control group ($p < 0.05$). In addition, GJD treatment significantly and dose-dependently decreased serum IL-6 levels and increased IGF-1 levels ($p < 0.05$) in the rats.

DISCUSSION

The high incidence, serious complications and dramatically decreased quality of life associated with osteoporosis are evidence of the severe effects of this disease in humans. Despite the pharmacological and clinical advantages of HRT as a widely accepted therapeutic strategy for osteoporosis, serious side effects of long-term application have also been reported. Therefore, development of new preventive and therapeutic drugs for osteoporosis is of critical importance. Interestingly, Chinese medicinal herbal extracts have been extensively investigated for their pharmacological effects, especially as they relate to preservation of bone integrity [17].

Decreased BMD is one of the major factors that jeopardize the strength of the bone, thereby resulting in increased susceptibility to fractures. Thus, BMD measurement can best predict risk of fracture [18]. The results obtained 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 GJD dose-dependently and significantly blocked decreases in BMD. Although BMD is among the strongest predictors of resistance to facture, empirical observations and theoretical analyses have shown that the biomechanical properties of bone

and trabecular microarchitecture influence trabecular bone strength as well [19].

Estradiol plays an important role in human sclerotin. When female serum estradiol level decreases significantly, osteoporosis occurs [20]. Compared to the OVX group, GJD treatment significantly increased serum E2, FSH and LH levels in a dose-dependent manner in the osteoporotic rats. Studies have shown that high doses or chronic administration of IL-6 in rats or mice caused increased degradation of proteins in skeletal muscle, although the normal levels of IL-6 have been proven hypertrophic [21]. Indirect effects of IL-6 on IGF-1 signaling have also been reported. Increased circulating levels of IL-6 are associated with significantly reduced serum IGF-1 levels and elevated expression of muscle SOCS3 mRNA [22]. These suggest the role of IL-6 as a negative regulator of IGF-1 signaling. Compared with the OVX group, GJD treatment significantly decreased serum IL-6 level and increased IGF-1 level in a dose-dependent manner in the rats.

CONCLUSION

The results obtained in the present study indicate that GJD can prevent OVX-induced osteoporosis in rats without hyperplastic effects on the uterus. This suggests that GJD has promising potential for future use in treating post-menopausal osteoporosis.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors 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 them.

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REFERENCES

1. *NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and therapy. Osteoporosis prevention, diagnosis, and therapy. J Am Med Ass 2001; 285: 785-795.*
2. *Eastell R, Black DM, Boonen S. Effect of once-yearly zoledronic acid five milligrams on fracture risk and change in femoral neck bone mineral density. J Clin Endo Metabol 2009; 94: 3215-3225.*
3. *Burge R, Dawson-Hughes B, Solomon DH. Incidence and economic burden of osteoporosis-related fractures in the United States 2005-2025. J Bone Min Res 2007; 22: 465-475.*
4. *Omsland TK, Emaus N, Tell GS. Mortality following the first hip fracture in Norwegian women and men. A NOREPOS study. Bone 2014; 63: 81-86.*
5. *Marcus R. An expanded overview of postmenopausal osteoporosis. J Mus Neuronal Int 2002; 2: 195-197.*
6. *Sugerman DT. JAMA patient page. Osteoporosis JAMA 2014; 311: 104-105.*
7. *Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporosis Inter 2006; 17: 1726-1733.*
8. *Stevenson JC. Justification for the use of HRT in the long-term prevention of osteoporosis. Maturitas 2005; 51: 113-126.*
9. *Prelevic GM, Kocjan T, Markou A. Hormone replacement therapy in postmenopausal women. Minerva Endocrinologica 2015; 30: 27-36.*
10. *Gray S. Breast cancer and hormone-replacement therapy: the Million Women Study. Lancet 2013; 362: 1332-1333.*
11. *Orija IB, Mehta A. Hormone replacement therapy: current controversies. Clinical Endocrinol 2013; 59: 657-658.*
12. *Lacey JV, Mink PJ, Lubin JH. Menopausal hormone replacement therapy and risk of ovarian cancer. J Ame Med Asso 2012; 288: 334-341.*
13. *Rossouw JE, Anderson GL, Prentice RL. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. J Ame Med Assoc 2012; 288: 321-333.*
14. *Lee JK, Kim KW, Choi JY. Bisphosphonates-related osteonecrosis of the jaw in Korea: a preliminary report. J Korean Asso of Oral and Maxi Surgeons 2013; 39: 9-13.*
15. *Riggs BL, Hodgson SF, O'Fallon MW. Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. New England J Med 1990; 322: 802-809.*

16. European Commission [homepage on the internet]. Directive 2010/63/EU on the protection of animals used for scientific purposes [cited 2013 Jan 16]. Available from: http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm.

17. Wronski TJ, Dann LM, Scott KS. Endocrine and pharmacological suppressors of bone turnover protect against osteopenia in ovariectomized rats. Endoc 2009; 125: 810-816.

18. Turner RT, Vandersteenhoven JJ, Bell NH. The effects of ovariectomy and 17 beta-estradiol on cortical bone histomorphometry in growing rats. J Bone Min Res 2010; 2: 115-122.

19. Dai SQ, Yu LP, Shi X, Wu H, Shao P, Yin GY. Serotonin regulates osteoblast proliferation and function in vitro. Braz. J. Med Biol. Res. 2014; 47: 759-765.

20. Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell and more. Endocr. Rev. 2013; 34: 658–690.

21. Carson JA, Baltgalvis KA. Interleukin 6 as a key regulator of muscle mass during cachexia. Exerc. Sport. Sci. Rev. 2010; 38: 168–176.

22. Munoz-Canoves P, Scheele C, Pedersen BK, Serrano AL. Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword? FEBS 2013; 280: 4131–4148.