

The Osteoprotective Effect of Zhuanggu-Guanjie Pill in Orchidectomized Mice

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Abstract. To verify the osteoprotective effect of Zhuanggu-Guanjie (ZGGJ) in male mice. The male mice were randomly divided into 6 groups: control group (sham-operation), model group (orchidectomy group), three ZGGJ groups at doses of 0.77, 1.54 and 3.08g/kg, as well as Alendronate sodium group as positive control. ZGGJ was intragastric administration daily for 8 weeks until mice were executed followed anesthesia. ZGGJ can ameliorate osteoporosis with increasing bone strength and improve trabecular bone microstructure which was proved by the Micro-CT scan. Serum osteocalcin (OC), alkaline phosphatase (ALP) and tartrate resistant acid phosphatase (TRACP) were examined, and the decreased OC implied the reduction of bone destruction. These results suggest that ZGGJ pill has significant therapeutic effect on osteoporosis in orchidectomized mice.

Introduction

Osteoporosis is a systemic skeletal disease, which is characterized by reduction of bone density and deterioration of the micro-architecture in the bone. What's more, Osteoporosis is prone to fracture. It increase morbidity and the mortality in the elderly significantly [1]. In addition, it negatively affects the patient's quality of life, as well as imposes a huge socioeconomic burden [2]. In 2010, the total cost for treatment of osteoporotic fracture was \$9.45 billion, and it will increase to \$25.43 billion by 2050 in China [3]. Despite osteoporosis occurs mainly in women, especially in postmenopausal women, there is also a certain incidence of osteoporosis in men [4-5]. Lifetime risk of osteoporotic fracture is very high which reaches 40-50% in women and 13-22% in men [6]. According to clinical reports [7], Zhuanggu-Guanjie pill (ZGGJ) presented a significant effect in treating osteoporosis, which indicate potential application of this drug in treating osteoporosis. Therefore, this study is to examine the therapeutical role of ZGGJ in treating osteoporosis in the male animal model.

Materials and Methods

Drugs

ZGGJ was purchased from Shenzhen CRC san-jiu pharmaceutical co., Ltd. Alendronate Sodium tablet is product of Merck Sharp & Dohme Italia PSA (Italy).

Animals and Treatment.

ICR male mice, SPF, weighting 20~25g, 6 weeks old, were purchased from Beijing HFK biotechnology Co., LTD (Beijing, China). The mice were feed in experimental animal center of Tianjin University of Traditional Chinese medicine, with the temperature 20~25°C, humidity 40~60%. Mice eat a standard diet and drink water. The animals experiments were permitted by the Laboratory Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (Permit Number:TCM-LAEC2015013).

The mice were orchidectomized to establish experimental osteoporosis [8]. After 6 weeks, the surviving mice were randomly divided into 6 groups: control group (sham-operation), model group (orchidectomy), positive drug group (orchidectomy + alendronate sodium), ZGGJ groups (0.77g/kg, 1.54g/kg and 3.08g/kg). Mice were intragastrical administration once a day for 8 weeks. The control group and the model group mice were treated with the same volume of water. After the final treatment, the mice were anesthetized and then sacrificed. Blood was collected to detect serum indicators, tibia to be detected bone density, and femur to be tested bone strength.

Measurement of Serum OC, ALP, TRACP.

According to the operating instruction, serum OC was measured using ELISA kits (Cloud-clone Co. Ltd, Wuhan, China). Serum ALP and TRACP were measured using ALP kits (BIOSINO Biotechnology Co., Ltd.) and TRACP kits (Beyotime Institute of Biotechnology, Jiangsu, China).

Trabecular Bone Histomorphometry

Right tibia was preserved in 75% ethanol which was replaced once every day and changed three times. After the final replacement ,tibia was preserved in anhydrous ethanol. Trabecular bone structure was performed using viva CT40 (SCANCO Medical AG, Zurich, Switzerland). Bone connectivity density (Conn.D), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) was calculated by plate model, and the scan region was below the epiphyseal growth plate of proximal tibia, extending 1 mm towards the distal direction.

Bone Strength Test.

Right femur was used to test bone strength in mode 1 using YLS-16A small animal bone strength analyzer (Jinan Yiyan Technology Co. Ltd., Jinan, China), and the result is the femur maximum load capacity indicating the maximum force (showed as gramme) being applied to the femur until it was fractured.

Statistical Analysis.

Data are formulated as the MEAN \pm SEM. Statistical analysis was performed according to T-test. The probability values of $p < 0.05$ were considered as significance.

Results

Compared with control group, the TRACP and ALP do not change with ZGGJ administration. On the other hand, serum ALP in treatment groups was higher than that of model group at doses of 1.54g/kg and 3.08 g/kg ($P<0.01$). Serum OC in model group increased significantly upon orchidectomy ($P<0.05$), but it dropped significantly with administration of ZGGJ at 1.54g/kg for 8 weeks ($P<0.05$). (Table1)

Table 1. Effects of ZGGJ on serum indicators

group	ALP(U/L)	TRACP(U/L)	OC (pg/ml)
Con	27.30±9.10	19.87±7.22	177.69±45.13
Mod	30.91±8.98	21.96±7.28	220.35±25.08 [#]
As	35.17±11.19	25.11±8.76	245.75±28.82
ZGGJ 0.77g/kg	42.50±20.41	20.51±5.27	218.75±26.99
ZGGJ 1.54g/kg	51.18±19.84 ^{**}	25.35±8.14	187.47±37.41 [*]
ZGGJ 3.08g/kg	44.73±13.08 ^{**}	29.46±11.43	208.45±47.65

Note: Con, control group; Mod, model group; AS, Alendronate Sodium group; ZGGJ, Zhuanggu-Guanjie pill. [#] $p<0.05$ compared with control group; ^{*} $p<0.05$, ^{**} $p<0.01$ compared with model group.

After drug treatment for 8 weeks, when the mice tibia was scanned by micro CT, it could be found that the trabecular bone reduced significant in model group and then it restored after treatment (Fig. 1A). Compared with the control group, the Tb. N and Conn. D of model group decreased significantly ($P<0.01$), while the Tb. Th, Tb. Sp significantly increased ($P<0.01$); Compared with the model group, the Conn. D, Tb. N of the treatment groups significantly increased, and the Tb. Sp decreased significantly ($P<0.01$). The Tb. Th of the alendronate group reduced ($P<0.05$), and ZGGJ group had a significant decrease ($P<0.01$) (see Fig. 1B).

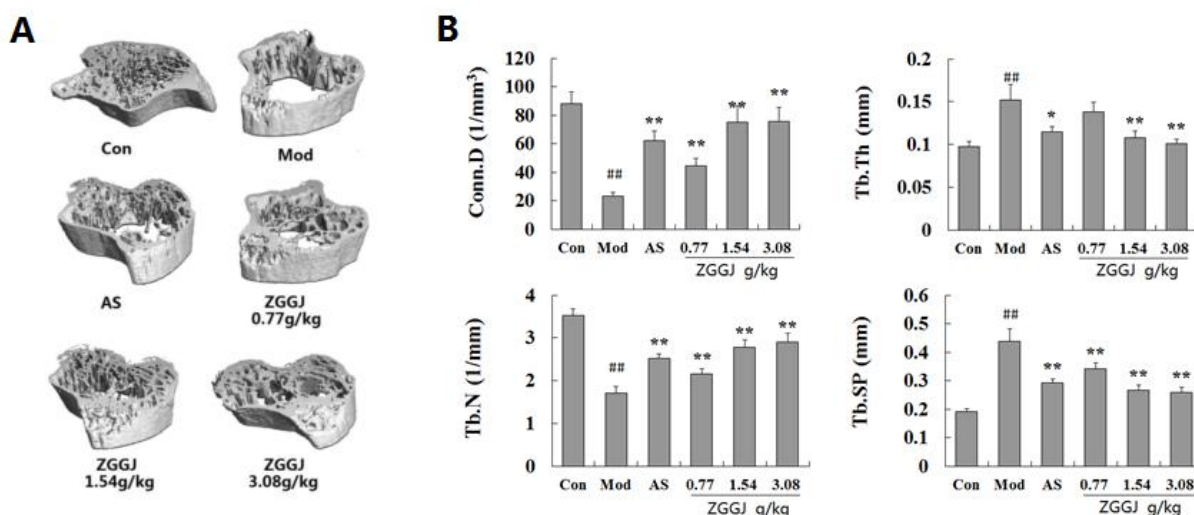


Figure 1. Trabecular bone analysis by Micro-CT.

Note: (A) The CT-scanning images. (B) Effects of ZGGJ on Conn. D, Tb. N, Tb. Th and Tb. Sp.

Con, control group; Mod, model group; AS, Alendronate Sodium group; ZGGJ, Zhuanggu-Guanjie pill. ^{##} $p<0.01$ compared with control group; ^{*} $p<0.05$, ^{**} $p<0.01$ compared with model group

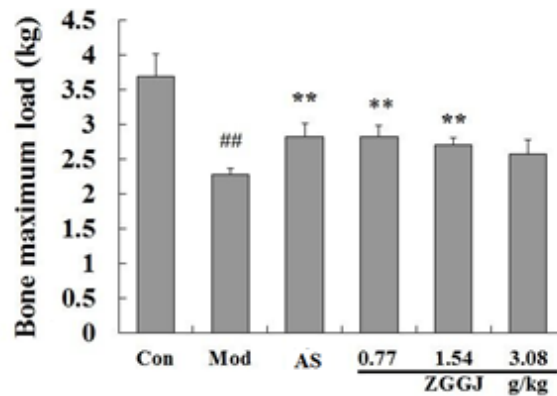


Figure. 2. Effects of ZGGJ on bone strength.

Note: Con, control group; Mod, model group; AS, Alendronate Sodium group; ZGGJ, Zhuanggu-Guanjie pill. ## $p < 0.01$ compared with control group; ** $p < 0.01$ compared with model group

The femur maximum load of the model group was significantly lower than that of control group ($P < 0.01$). After drug treatment with different dosage, the capacity of femur maximum load enhanced. Moreover, the femur maximum load in alendronate group and ZGGJ groups (0.77 g/kg and 1.54 g/kg) increased significantly compared with the model group ($P < 0.01$) (Fig. 2).

Conclusion

Compared with rat model, mice model is more time effective, it took only 8 weeks to observe obvious osteoporosis after orchidectomy. The main bone manifestation include decreased trabecular bone density and number and increased gap between them, which represent osteoporotic rats [9].

OC is a serum marker secreted by osteoblasts that reflects bone conversion rate. Serum OC of osteoporotic patients would increase significantly [10]. Our data revealed that ZGGJ can reduce the level of serum OC, especially when using medium dose at 1.54 mg/kg.

After treated with ZGGJ, the Conn.D and Tb.N of mice increased significantly, while Tb.Sp decreased obviously. By contrast, Tb.Th was intact upon administration of ZGGJ. This shows that ZGGJ has a certain effect on reconstruction of bone trabecular structure, therefore a therapeutic effect on osteoporosis.

Osteoporotic fracture is the main complication of osteoporosis [11]. So the objective of osteoporosis treatment is to reduce the risk of bone fracture. The bone maximum load directly reflects the capability of resisting fracture. While the bone maximum load increased with administration of ZGGJ. Although there is no dose-effect relationship, it has a good therapeutic effect in human within the equivalent dose.

In summary, ZGGJ has a certain therapeutic effect on osteoporosis of male mice caused by orchidectomy, so it provided experimental basis for its clinical application in the treatment of men osteoporosis.

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