The Use of a Noninvasive and Nondestructive Method, Microcomputed Tomography, to Evaluate the Anti-Osteoporotic Activity of Erxian Decoction, a Chinese Medicinal Formula, in a Rat Model of Menopausal Osteoporosis

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Abstract

Aim of the study: The anti-osteoporotic activity of Erxian Decoction, a Chinese medicinal formula, in a rat model of menopausal osteoporosis was evaluated by microcomputed tomography (microCT).

Materials and methods: Menopause causes a decline in both endocrine function and bone mineral density in human. In this study, 20-month-old female Sprague-Dawley-rats (SD-rats) with a low serum estradiol level and bone mineral density were employed. The anti-osteoporotic activity of EXD was assessed by the determination of trabecular material bone mineral density at the L2 mid-vertebral body after treatment. Serum estrogen levels were also determined to assess the effect of EXD on the endocrine status.

Results: Results revealed a significant elevation in serum estradiol level and trabecular bone mineral density at the L2 mid-vertebral body in the EXD-treated menopausal rat model.

Conclusions:

The results obtained from the present investigation revealed that the EXD had anti-osteoporotic activity as evidenced by an increase of serum estradiol level and bone mineral density.

Keywords- *Microcomputed Tomography, osteoporosis, Erxian Decoction, Chinese medicine, estradiol and menopause.*

1. Introduction

Menopause is the period during which estrogen secreted by the ovaries gradually declines and patients suffer from much higher incidences of body and mental changes, such as oesteoporsis [3]. It is also believed the low levels of estrogen in patients are responsible for the peri-menopausal symptoms, including osteoporosis [3]. Menopause is normally treated by hormone replacement therapy. However, such treatment may increase the incidence of different side effects, i.e. breast cancer and stroke [2]. Therefore, scientists are seeking safer and more effective alternatives [1].

Erxian Decoction (EXD), a popular Chinese medicinal formula, has been clinically used in the treatment of menopausal syndrome for more than 50 years. Nian et al. (2006) and Qin et al. (2008) reported that EXD and its chemical constituents possessed antiosteoporotic activity in ovariectomized rats and it increased the serum estradiol level [3, 4]. The L2 mid-vertebral body has been used clinically for assessment of osteoporosis [5]. However, the utility of micro-CT imaging in evaluating the efficacy of the Chinese medicinal formula, EXD, on anti-osteoporosis regimen has not been reported. In this study, we propose to evaluate the anti-osteoporotic activity of EXD by measuring the trabecular material bone mineral density at the L2 mid-vertebral body.

2. Materials and methods:

2.1 Herbal materials

Six herbal materials, including *Curculigo orchioides, Epimedium brevicornum, Morinda officinalis How, Angelica sinensis, Phellodendron chinense and Anemarrhena asphodeloides* (bearing voucher numbers. CME140706-03, CME150906-11, CME050406-02, CME090706-12, CME100606-09, and CME180806-01, respectively) were collected from various sources and their identities were confirmed by Professor Zhengtao Wang, Department of Pharmacognosy, China Pharmaceutical University. The voucher specimens were kept in the China Pharmaceutical University.

2.2 Preparation of EXD

One kilograms of a mixture of the six medicinal materials was extracted by decocting it with distilled water, 10:1 (v/w), at 100℃ for 1 h. The extraction was repeated twice. The

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filtrates were lyophilized in a freeze drier (Labconco, Freezone), and kept at 4℃ for quality control and molecular studies. The yield of dried extract from the starting crude materials was 20%.

2.3 Animals

Twelve-month-old female Sprague–Dawley (SD) rats, with a low serum estradiol level and trabecular bone mineral density, were employed as a menopausal animal model in this study. Female SD rats, aged 8 months, were purchased from the Laboratory Animal Unit, the University of Hong Kong. The animals were housed in an air-conditioned room at an ambient temperature of 24℃ and 50-65% relative humidity under a 12 h light: 12 h dark photoperiod. They were acclimated for 4 months and their serum estradiol levels monitored before the experiment. The experiment had been approved by the Committee on the Use of Live Animals in Teaching and Research (CULATR) of Li Ka Shing Faculty of Medicine, the University of Hong Kong.

2.4 Drug administration and sera collections

Rats were randomly divided into four groups of 10 animals each. The Chinese medicinal EXD extract (0.76 and 1.52 g/kg) and the Western medicine Premarin capsule (31.25 mg/kg) (each capsule containing 0.3 mg of estrogen) were separately administered by the oral route, everyday for 6 weeks. The control group received orally an equal volume of water instead of EXD. At the end of the experiment, the rats were anesthetized by an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) dissolved in 0.9% saline. Their sera and L1-6 mid-vertebrae were collected and stored at -80℃ for further analysis.

2.5 Detection of serum estradiol level

The estradiol levels in serum samples were determined by using the electro-chemiluminescence immunoassay (Elecsys 1010; Roche Diagnostics), following the manufacturer's instructions.

2.6 Measurement of bone mineral density

The lumbar vertebrae in each group were harvested for trabecular bone mineral density assessment by microcomputed tomography (microCT). The isolated lumbar vertebrae from the four group of rats were placed inside a sample holder and the long axis aligned with the axis of rotation of the X-ray gantry. A scout view was obtained to identify the 2nd lumbar vertebra (L2). Then a set of 100 axial slices at a resolution of 21 μm was acquired at the L2 mid-vertebral body with 70 KV and 110 μA using an In Viva MicroCT 40 computed tomography system (Scanco Medical, Basserdorf, Switzerland). Volume rendering of these slices was performed to provide a three-dimensional (3D) image. The trabecular material bone mineral density was automatically evaluated using the built-in program of the microCT with direct 3D morphometry. One-way ANOVA was employed to find out any significant difference.

3. Result and discussion:

Our recent study showed that EXD with anti-osteoporosis can increase the trabecular bone mineral density at L2 mid-vertebral body [Fig 1A &4B]. Osteoporosis is one of the major menopausal symptoms. The trabecular bone mineral density at L2 mid-vertebral body has been used clinically for assessment of osteoporosis [5]. Estrogen deficiency is known to be an important contributing factor in the pathogenesis of osteoporosis. EXD treatment can increase the level of estrogen in old female rats [Table 1]. The loss of estrogen has been related to an increase in the bone remodeling rate which results in a negative bone balance. This finding demonstrated that EXD brought about a reversal of the negative bone balance with an increase in trabecular bone mineral density compared with the control. In addition, a significant linear trend effect was also demonstrated with the use of EXD [Fig 1D]. In conclusion, EXD exhibited an anti-resorptive effect, reduced the frequency of bone remodelling events and allowed a full degree of bone mineralisation. We are the first group to demonstrate that EXD treatment can increase trabecular bone mineral density at the L2 mid-vertebral body. Therefore, the trabecular bone mineral density will be used as a biomarker to assess the efficacies of the other Chinese herbs and their constituents for relieving menopausal osteoporosis.

4. Conclusion:

Twenty-month-old female SD rats prescribed with EXD showed a higher trabecular material bone mineral density at the L2 mid-vertebral body and serum estrogen level than the intact control. Results obtained from this study could provide a simple and authentic strategy for investigating menopausal osteoporosis that may open new alternatives for the treatment of menopausal osteoporosis.

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Figure and Table

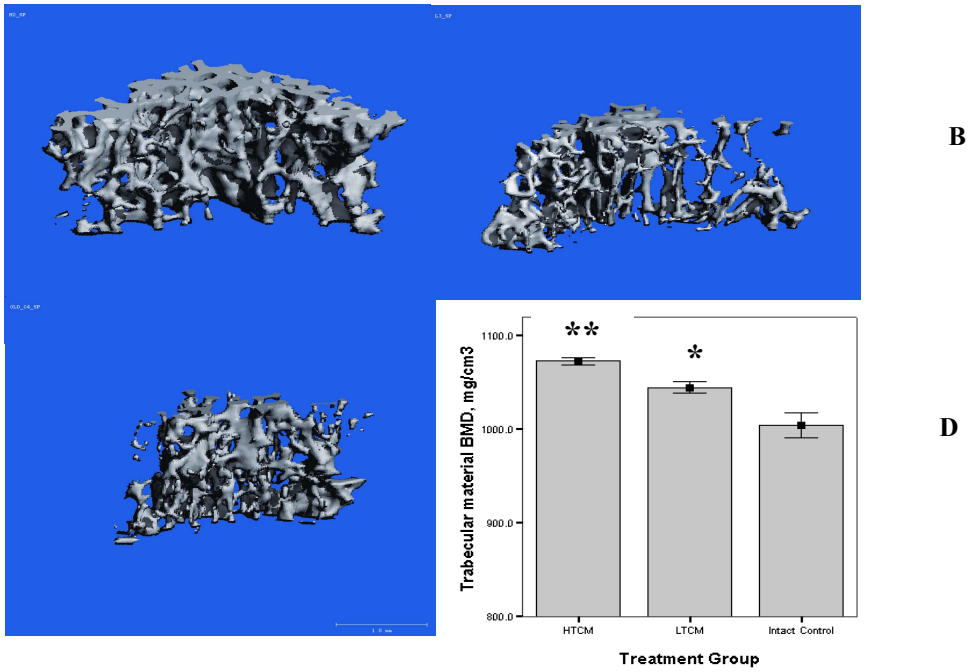

Fig 1. MicroCT scan of 20-month-Old SD rats showing L2 vertebral body trabecular bone from the various groups. **Fig 1A** (high dose EXD, 1.52 g/kg/day, 6 weeks); **Fig 1B** (low dose EXD, 0.76 g/kg/day, 6 weeks); **Fig 1C** (Intact control); **Fig 1D** After analysis, the high dose EXD (HTCM) had a trabecular BMD of 2.7 % and 6.8 % higher than the low dose EXD (LTCM) and intact control group, respectively, (** $P < 0.05$) whereas that of the LTCM group was 4.1% higher than the intact control (* $P = 0.005$). Analysis with ANOVA with LSD post hoc test. Ascending trend increase was shown from intact control to High dose EXD group.

Table 1

Effect of EXD on serum estradiol level (n=10, mean±SD)

Group	estradiol level (pg/ml)
Young rat group (3-month-old SD rats)	225.6±73.6
Control group (12-month-old SD rats)	58.9±21.2##
EXD-treated group (0.76 g/kg)	93.2±33.5*
EXD-treated group (1.52 g/kg)	170.2±54.6**

P<0.01 compared with young rat group; * *P<0.05*, ** *P<0.01* compared with old rat group. (The data in this table were published in Journal of Ethnopharmacology, [6])