

Electroacupuncture Promotes Insulin-Like Growth Factors System in Ovariectomized Osteoporosis Rats

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Abstract: Postmenopausal Osteoporosis (PMOP) is induced by the deficiency of estrogen in postmenopausal women. Electroacupuncture (EA) has been confirmed to be effective in clinic. We adopted ovariectomized osteoporosis model of rats to observe the role of EA in PMOP. Fifty female SD rats were divided randomly into 5 groups: intact (INT, $n = 10$), sham operation (Sham, $n = 10$), model ($n = 10$), estrogen (E, $n = 10$) and electroacupuncture (EA, $n = 10$). The bone mineral content (BMC) and the bone mineral density (BMD) were examined in lumbar_{1–6} and right thigh-bone, respectively, and estradiol (E₂), insulin-like growth factor I (IGF-I) and insulin-like growth factor binding proteins (IGF-BPs) were tested by RIA or ELISA. The results showed that BMC and BMD of lumbar 1–6 and right thigh-bone in PMOP model rats decreased markedly, while the level of serum E₂, IGF-I and IGF-BP1 were lower than in INT and Sham. However, EA could upgrade the contents of IGF-I and

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IGF-BP1 to increase BMD in PMOP rats, while no significant difference was seen in E group. Therefore, EA may promote IGF system to improve PMOP.

Keywords: Postmenopausal Osteoporosis; Insulin-Like Growth Factor System.

Introduction

Postmenopausal osteoporosis (PMOP) is one of the most prominent chronic diseases due to the sharp decline of estrogen in postmenopausal women. Therefore, the imbalance in bone remodeling caused by increased bone resorption and decreased bone formation takes place. In terms of the mechanisms for the age-related impairment in bone formation, a deficiency in growth hormone (GH)/insulin-like growth factor (IGF) system that occurs with age has been proposed to play a major role. Although a number of animal and clinical studies have provided experimental evidence for potential use of components in IGF system to increase bone mass, the clinical utility of the components to treat osteoporosis has not come to fruition because of our incomplete understanding of the regulation of production and actions of the components in IGF system (Kasukawa *et al.*, 2004).

Acupuncture has long been associated with homeostatic (Yin/Yang) regulation and known to possess effects such as facilitating the release of neuropeptides in the central nervous system (CNS), eliciting profound physiological effects and even activating self-healing mechanisms (Han, 2003). In clinic, acupuncture is widely adopted to treat the pain induced by PMOP effectively and successfully (Zhang and Liu, 2006), which is a useful alternative and complementary therapy. The results proved that acupuncture could improve the BMC and BMD, as well as various indexes related with PMOP. However, the scientific mechanism of acupuncture remains poorly defined. In the present study, we observed BMC and BMD by a dynamic filtration double-energy X-ray (DFDE-X) bone mineral density instrument, and tested the levels of serum estradiol (E₂), IGF-I, IGF-BP1 and IGF-BP3, which might provide the theoretical understanding in clinical treatment.

Materials and Methods

Animals and Experimental Protocol

Fifty 12-month-old female Sprague-Dawley rats (200–220 g), with regular 4-day estrous cycle were purchased from the Medical Experimental Animals Center of Fudan University (Shanghai, China). Animals were kept in group cages with *ad libitum* access to food and water, under the condition of controlled temperature at $22 \pm 1^\circ\text{C}$ and a schedule of 12/12 (light/dark). Rats were divided into 5 groups randomly as follows: intact (INT, $n = 10$), sham operation (Sham, $n = 10$), model (Model, $n = 10$), estrogen (E, $n = 10$) and electroacupuncture (EA, $n = 10$).

Ovariectomy was performed under anesthetic ether (inhalation anesthesia, Chinese Medical and Chemistry Co. China) on rats of the Model, E and EA groups. In sham animals, only the fat around ovaries was excised under the same anaesthesia. Then all rats were confirmed

by examination with daily exfoliative epithelia by vaginal smears for two sequential estrous cycles (about 8–9 days). INT and Sham showed the regular 4-day estrous cycle, while the ovariectomized rats had no daily change in exfoliative epithelia in vaginal smears. Rats were kept under same condition for 16 weeks. All experimental procedures were in strict accordance with the guidelines of the Shanghai Medical College of Fudan University.

Oral Administration and EA Treatment

Nilestriol (1 mg/tablet, Beijing Four-cycle Drug Manufactory, Batch Production No. 20021001) was dissolved into distilled water (0.01 mg/ml). Rats in E group were orally administered 0.9 ml/week/100 g (rat weight) of Nilestriol which was a conversion dosage for rats (Li *et al.*, 2007).

Rats in the EA group were treated by EA with bilateral “Xuanzhong (GB39)” and “Mingmen (DU 4)” acupoints for 30 min around 8:00–12:00 a.m., Q.D. for 4 weeks. The needles used were: diameter 0.22 mm and length 10 mm, inserted using standard insertion pressure. The principle of choosing points followed the standard for rats recorded in Experimental Acupuncturology (Lin and Wang, 1999). Xuanzhong (GB39) is located on the outside of the hind limb and about 5 mm superior to the top of the lateral malleolus. Mingmen (DU 4) is located inferior to the second lumbar vertebra. Needles were fixed to acupoints by rubber cement. Electric impulses were from the G6805-II therapeutic stimulator with a frequency of 2 Hz (120 turns/min) and an intensity of 1 mA to produce slight twitches in the limbs. All rats were conscious and only fixed gently by researchers’ hands. The rats did not show any pain or discomfort during the treatments. By the end of the last experiment, the animals were scanned by DFDE-X bone mineral density instrument under anaesthesia, and then sacrificed and the serum was extracted.

Measurement of BMC, BMD

BMC and BMD were assessed by DFDE-X bone mineral density instrument with an apparatus for small animals (Norland XR-36 USA) under anaesthesia. The scanning parameter is 1.0×1.0 mm, 60 mm/s, 12.00 cm, REV.3.9.3/2.1.0. Analysis was carried out with a box method for the lumbar spines and a random method for the right thigh. Average of the Lumbar_{1–6} vertebrae and right thigh were calculated.

RIA and ELISA of Blood Estrogen and IGFs Concentrations

At the time of sacrifice, along with the corresponding intact controlling animals, the blood samples (1 ml) were collected, during the period of diestrous. The plasma was separated by centrifugation and stored at -70°C until assayed. Concentration of blood estrogen was determined by double-antibody RIA kits purchased from the National Atomic Energy Research Institute (Beijing, China). The samples were assayed in duplicate, and all samples were assayed together. The sensitivity of the kit was 2 pg/ml, its intra- and inter-assay coefficients of variation was 4.74–7.7%. IGF-I, IGF-BP1 and IGF-BP3 were examined by ELISA

(Diagnostic Systems Laboratories, DSL, USA). The sensitivity of the kit for IGF-I, IGF-BP1 and IGF-BP3 was 30 ng/ml, 0.25 ng/ml and 0.04 ng/ml respectively. All procedures were strictly in accordance with standards and dictates of Fudan University.

Data Analysis and Statistics

All results were expressed as mean \pm SD and analyzed by the Statistical Package for the Social Sciences (SPSS) statistical software (version 11.5). The raw data was compared by using the one-way analysis of variance (ANOVA) and significance of difference was determined by the Student-Newman-Keuls *post hoc* test. Significance was set at $p < 0.05$ and $p < 0.01$ in the two-tailed testing.

Results

BMC and BMD of Lumbar₁₋₆ and Right Thigh

Twelve weeks after ovariectomy, several rats were selected randomly and examined for BMC and BMD to determine the success of the models compared to INT. Thereafter, after 4 weeks treatment with estrogen or EA, all rats were examined under the DFDE-X bone mineral density instrument. BMC and BMD of lumbar₁₋₆ and the right thigh in the model group appeared to be significantly lower than in INT and Sham ($p < 0.05$, $p < 0.01$, respectively); however, estrogen slightly increased the BMC and BMD of the right thigh while EA tended to enhance the BMD of lumbar₁₋₆ (Fig. 1).

Estrogen and IGFs Level in Serum of INT, Sham, Model, E and EA Rats

Compared to INT, the level of serum estradiol decreased significantly in Model and EA groups ($p < 0.01$) while no difference was seen in Sham and E (Table 1). However, the estrogen level in E group was higher than that of Model ($p < 0.01$). As shown in Table 2, the IGF-I and IGF-BP1 levels were lower markedly in Model group in contrast to those in INT and Sham. Both E and EA elevated the IGF-I level and EA also enhanced the IGF-BP1 content. Although the IGF-BP3 also tended to change in different groups, no significant difference was found. It indicates that estrogen could effectively increase the serum estradiol in PMOP rats, while EA increased the level of serum IGF-I and IGF-BP1 in PMOP rats.

Discussion

PMOP is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue leading to enhanced bone fragility and a consequent increase in the fracture risk with age (Mohan and Baylink, 1999). The pathogenesis of postmenopausal osteoporosis is not known in detail but widely understood that the rate of bone resorption is greater than the rate of bone formation, which results in net bone loss. The early identification of postmenopausal women at risk of fracture, followed by effective therapeutic intervention, can

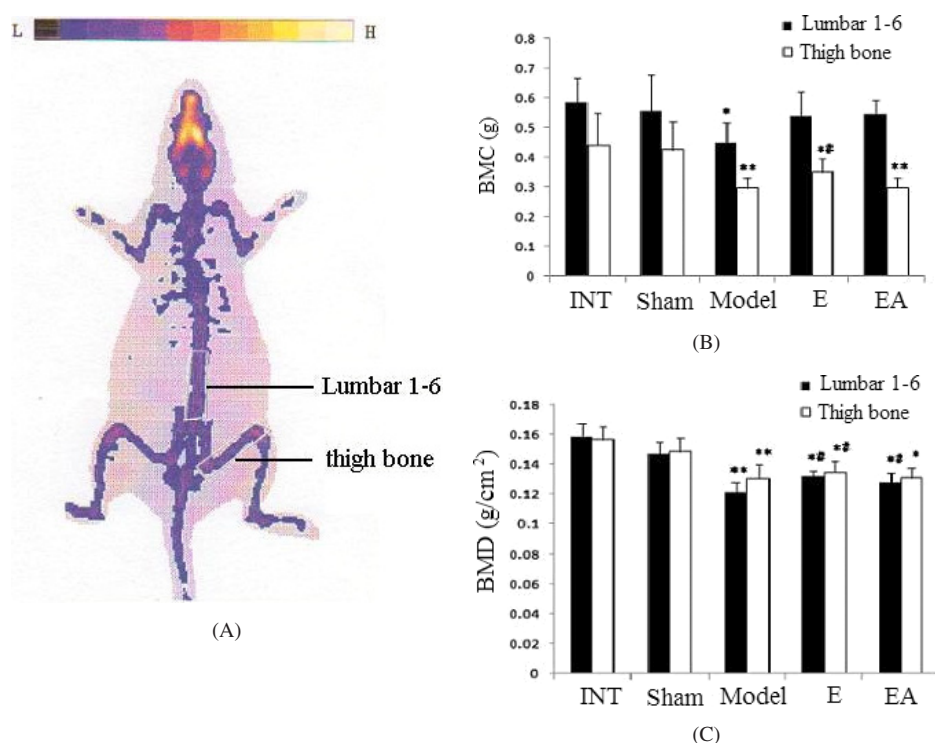


Figure 1. (A) The scanogram of BMC and BMD of rats examined by DFDE-X bone mineral density instrument (Norland XR-36 USA) under anaesthesia. Rats were laid in prone position. BMC and BMD of lumbar₁₋₆ and thigh bone were calculated automatically by software. (B) BMC of lumbar 1-6 and thigh bone (right) were expressed as the mean with SD bar in each column indicated in the lower panel. * $p < 0.05$ vs. INT; ** $p < 0.01$ vs. INT and # $p < 0.05$ vs. Model. (C) BMD of lumbar₁₋₆ and thigh bone (right) were expressed as the mean with SD bar in each column indicated in the lower panel. * $p < 0.05$ vs. INT; ** $p < 0.01$ vs. INT; # $p < 0.05$ vs. Model.

Table 1. The Estradiol Contents in Serum of INT, Sham, Model, E and EA Groups

Groups	n	E ₂ (pg/ml)
INT	10	106.67 ± 16.10
Sham	9	91.87 ± 21.75
Model	9	47.37 ± 10.81**
E	8	97.93 ± 10.92##
EA	10	41.23 ± 18.26**

(X ± S) ** $p < 0.01$ vs. INT, ## $p < 0.01$ vs. Model.

substantially improve outcome in patients. Effective pharmacologic treatments are available that have demonstrated reductions in the risk of vertebral and nonvertebral fractures. Some therapies, such as selective estrogen receptor modulators and bisphosphonates, are able to act early in the course of treatment to reduce this risk of vertebral and nonvertebral

Table 2. The IGF-I, IGF-BP1 and IGF-BP3 Contents of Serum in INT, Sham, Model, E and EA Groups

Groups	n	IGF-I(ng/ml)	IGF-BP1(ng/ml)	IGF-BP3 (ng/ml)
INT	10	4259.68 ± 378.98	35.26 ± 8.97	19.43 ± 3.23
Sham	9	3766.90 ± 91.88	35.78 ± 5.95	19.80 ± 0.97
Model	9	2881.67 ± 520.56**	15.79 ± 5.66**	18.28 ± 2.82
E	8	4078.60 ± 478.72##	24.05 ± 9.23	17.90 ± 0.48
EA	10	4401.13 ± 724.52##	35.78 ± 11.37##	17.53 ± 0.64

(X±S) **p < 0.01 vs. INT, *p < 0.05 vs. INT, ##p < 0.01 vs. Model.

fractures (Levine, 2007). However, the results of the largest hormone replacement therapy (HRT) randomized clinical trial by the Women’s Health Initiative (WHI), indicated that long-term use of estrogen plus progestin HRT (EPRT) could increase cancer risk and might even increase cardiovascular disease risk (Anderson *et al.*, 2003). Meanwhile, a number of epidemiological studies have proven the relationship between HRT and the risk of cancer of female reproductive organs. The available evidence indicated that the risk of breast or endometrial cancer was increased among HRT users, and the risk increased with increasing duration of use (Collaborative Group on Hormonal Factors in Breast Cancer, 1997; Beral and Reeves, 2005). Furthermore, patients usually seek acupuncture treatment because of the pain induced by under diagnosed and under treatment of osteoporosis. Acupuncture is also regarded as a preventive treatment strategy for bone loss (Xu *et al.*, 2005). Maras (1996) reported that electroacupuncture plus auriculo-acupuncture were adopted to treat a lady with osteoporosis. The patient fully recovered from the serious back pain after nine treatments and BMD increased significantly after a 7-month treatment. Liu adopted the acupoints on the Governor Vessel, such as Baihui (GV20), Dazhui (GV14), Zhiyang (GV9), Mingmen (GV4). Four or six associated acupoints were chosen from Guanyuan (CV4), Qihai (CV6), Shenshu (BL23), Pishu (BL20), Xuanzhong (GB39), Taixi (KI3), Zhusanli (ST36), Sanyinjiao (SP6) and Cuiiao (BL32), leaving the needles inserted for 40 min after “de qi,” using reinforcing manipulation, once a day. Meanwhile, moxibustion was used at 2–3 acupoints for 15 min. The BMD of the ulna, radius, vertebrae and pelvis were examined before the treatments and three months after treatments (Liu *et al.*, 2000). The result showed that acupuncture might be an effective alternative and complementary therapy for pharmacologic treatment as a part of a more proactive approach to treating osteoporosis (Ouyang *et al.*, 2002).

Previous basic and clinical studies implied that there was a significant role for insulin-like growth factor-I (IGF-I) in BMD (Niu and Rosen, 2005). Recently, polymorphisms upstream of the P1 promoter region in the human IGF-I gene had been found to be associated with serum levels of IGF-I, BMD and fracture risk in various ethnic groups. Genomic engineering resulting in IGF-I deficient mice, and mice with targeted over-expression of IGF-I reinforced the essential role of IGF-I in bone development at both embryonic and postnatal stages. Thus, it was apparent that significant new insights into the role of the IGF-I gene in bone remodeling occur through several distinct mechanisms. Molecular dissection of the IGF regulatory system and its signaling pathway in bone may reveal novel therapeutic targets for the treatment of osteoporosis.

Of the six IGF-I binding proteins, IGF-BP1 and IGF-BP3 modulate most of IGF-I biological activity. Evidence also demonstrated the important regulatory roles for IGF BPs, both augment and inhibit IGF effects (Conover *et al.*, 1996; Chevallier *et al.*, 1998). IGF-BP3 binds more than 90% of circulating IGF-I, and in association with an acid-labile subunit, forms a stable ternary complex of high molecular weight (150 kDa), which prolongs IGF-I half-life and prevents IGF-I from crossing the capillary walls. IGF-I also forms smaller binary complexes with other BPs, mainly IGF-BP1 (30–40 kDa) which can cross capillaries, thereby facilitating the transit of IGF-I to target tissues (Sharf *et al.*, 1996). In our study, EA might increase IGF-I and IGF-BP1 and promote the binding of IGF-I and IGF-BP1 to enhance BMD in PMOP; whereas estrogen mainly affected E_2 and estrogen receptors (ERs). Estrogen not only is important for reproductive tissue functions but also influences several peripheral tissues including bone and cardiovascular tissue. The protective nature of estrogen on bone density in young and aged animals is well-established (Loose and Stancel, 2005). This notion is further supported by the discovery of ERs in the peripheral tissues in several species (Deroo and Korach, 2006). Therefore, EA might have a unique mechanism to suppress PMOP in contrast with estrogen.

Acupuncture has long been associated with homeostatic (Yin/Yang) regulation and acupuncture therapy used in reproductive and metabolic medicine today has been established in the West over the last decade by the supporting solid scientific data (Han, 2003; Ulett *et al.*, 1998). We had observed that EA may significantly increase the blood level of E_2 in ovariectomized rats (Zhao *et al.*, 2003). However, in this study, we did not see the effect of EA on E_2 in PMOP rats, maybe because 12-month-old female rats were used and experienced ovariectomy. Furthermore, it suggested that EA might increase BMD in PMOP rats via the IGF system instead of estrogen, which explain why less side-effects and cancer risk occurs with EA.

We speculated that GH, estrogen receptors (ERs), immune and metabolic regulation were involved in the mechanisms of EA on the IGF system. Initially, electrical stimulation of 20 pulses/sec for 20 min could induce an elevation of plasma GH; however, no change was found in the plasma level of ACTH at 20 min after the needle removal. The mechanism of EA in inducing GH secretion may engage the brain endorphin and serotonergic, catecholaminergic systems. The results of ACTH suggested that EA did not induce stress and the increased level of GH may be due to specific effects of electrical stimulation on the neuroendocrine processes (Debrececi, 1991). GH could activate the promoter of exon 2 of IGF-I to stimulate the IGF-I transcription in the blood of the liver, as well as activate the promoter of exon 1 to stimulate the IGF-I transcription in bone.

Subsequently, even though there is no elevated E_2 level induced by EA, acupuncture for women with postmenopausal syndrome was found to be effective in increasing ERs and ER mRNA in hypothalamus and peripheral organs and regulating neuro-endocrine-immune-metabolic network in patients and aging rats (Yu, 2004; Chen, 1997). ERs activation might be one of the reasons for the synthesis of IGF-I.

Moreover, EA could improve the body immune and metabolic functions. Interleukin-6, transformation growth factor- β , bone morphogenetic protein and so on might contribute to the effect of EA on IGF system (Rosen, 2004). However, all these speculations need to be proven in further studies.

In conclusion, EA might increase the contents of IGF-I and IGF-BP1 to increase the BMD in PMOP rats and have a different effect pathway compared with estrogen.

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References

- Anderson, G.L., H.L. Judd, A.M. Kaunitz, D.H. Barad, S.A.A. Beresford, M. Pettinger, J. Jiu, S.G. McNeeley and A.M. Lopez. Effects of estrogen plus progestin on gynecologic cancers and associated diagnostic procedures: the Women's Health Initiative randomized trial. *J. Am. Med. Assoc.* 290: 1739–1748, 2003.
- Beral, V.D.B. and G. Reeves. Million Women Study Collaborators. Endometrial cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 365: 1543–1551, 2005.
- Chen, B.Y. Acupuncture normalizes dysfunction of hypothalamic-pituitary-ovarian axis. *Acupunct. Electrother. Res.* 22: 97–108, 1997.
- Chevallier, B., A. Lagarde, H. Degrelle, J. Belaisch-Allart, P. Giraudet and J.P. Gallet. Insulin-like growth factor binding protein 1 level in amniotic fluid: correlation with birth weight. *Biol. Neonate* 73: 404–406, 1998.
- Conover, C.A., J.T. Clarkson and L.K. Bale. Factors regulating insulin-like growth factor-binding protein-3 binding, processing, and potentiation of insulin-like growth factor action. *Endocrinology* 137: 2286–2292, 1996.
- Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. *Lancet* 350: 1047–1059, 1997.
- Debrececi, L. The effect of electrical stimulation of the ear points on the plasma ACTH and GH level in humans. *Acupunct. Electrother. Res.* 16: 45–51, 1991.
- Deroo, B.J. and K.S. Korach. Estrogen receptors and human disease. *J. Clin. Invest.* 116: 561–570, 2006.
- Han, J.S. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. *Trends Neurosci.* 26: 17–22, 2003.
- Kasukawa, Y., N. Miyakoshi and S. Mohan. The anabolic effects of GH/IGF system on bone. *Curr. Pharm. Des.* 10: 2577–2592, 2004.
- Levine, J.P. Effective strategies to identify postmenopausal women at risk for osteoporosis. *Geriatrics* 62: 22–30, 2007.
- Li, L.R.Y. Feng, Y.L. Tan, Y.X. Liu, Y. Zhang, X.P. Wu, and M.L. Zhang. Effect on serum IGF-I and E₂ in postmenopausal rats by GNAT. *J. Hubei Tradit. Chin. Med.* 29: 5–6, 2007 (in Chinese).
- Lin, W.Z. and P. Wang. *Experimental Acupuncturology*. Shanghai Science and Technology, Shanghai, China, 1999, pp. 288–291.
- Liu, G.X. and D.Z. Zhang. Clinic report of 28 cases of senile osteoporosis by acupuncturing governor vessel. *Chin. Acupunct. Mox.* 20: 529–530, 2000 (in Chinese).
- Loose, D.S. and G.M. Stancel. Estrogens and progestins. In: Brunton L.L. (ed.) *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. McGraw-Hill, New York, 2005, pp. 1541–1571.
- Maras, I. Spontaneous osteoporosis treated by electroacupuncture. *Foreign. Med. Tradit. Chin. Med.* 18: 51, 1996 (in Chinese).
- Mohan, S. and D. J. Baylink. *The Aging Skeleton — Role of Growth Hormone/Insulin-Like Growth Factor Axis*. Elsevier Publishing, 1999, pp. 209–219.

- Niu, T. and C.J. Rosen. The insulin-like growth factor-1 gene and osteoporosis: a critical appraisal. *Gene* 361: 38–56, 2005.
- Ouyang, G., L. Wang, D. Wang, T. Zhuo and Z. Shen. The effect of acupuncture on bone mineral density in postmenopausal women. *J. Tradit. Chin. Med.* 22: 9–11, 2002.
- Rosen, C.J. Insulin-like growth factor I and bone mineral density: experience from animal models and human observational studies. *Best Pract. Res. Clin. Endocrinol. Metab.* 18: 423–425, 2004.
- Sharf, J.G., F. Schmitz, J. Frystyk, C. Skjarbaek, H. Moesus, W. Blum, G. Ramadori and H. Hartmann. Insulin-like growth factor-1 serum concentration and pattern of insulin-like growth factor-1 binding proteins in patients with liver diseases. *J. Hepato.* 25: 689–699, 1996.
- Ulett, G.A., S. Han and J.S. Han, Electro-acupuncture: mechanisms and clinical application. *Biol. Psychiatry* 44: 129–138, 1998.
- Xu, H., D. Lawson, A. Kras and D. Ryan. The use of preventive strategies for bone loss. *Am. J. Chin. Med.* 33: 299–306, 2005.
- Yu, J. Integrated traditional Chinese and western medicine should make new contribution to the reproductive health of women. *Acta Integr. West. Chin. Med. (Zhong Xi Yi Jie He Xue Bao)* 2: 83–85, 2004 (in Chinese).
- Zhang, Z.L. and Z.H. Liu. The new progressing in osteoporosis. *Chin. J. Osteoporos.* 12: 107–112, 2006 (in Chinese).
- Zhao, H., Z.Z. Tian and B.Y. Chen. An important role of corticotropin-releasing hormone in electroacupuncture normalizing the subnormal function of hypothalamus-pituitary-ovary axis in ovariectomized rats. *Neurosci. Lett.* 349: 25–28, 2003.

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