

仙杞煎液对实验性骨质疏松大鼠骨密度、白介素 6 和血浆酸性磷酸酶的影响

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摘要 目的:探讨仙杞煎液对大鼠实验性骨质疏松症的治疗作用及其作用机理。方法:选用 7 月龄 SD 雌性大鼠 72 只,随机分为 2 组。手术组 60 只行双侧卵巢切除术,假手术组 12 只行伪手术,复制成绝经后骨质疏松大鼠模型。将手术组随机分为模型组、西药组、仙杞煎液低、中、高剂量组,连同假手术组共 6 组按相应剂量灌胃给药。60 天后摘眼球取血后将大鼠处死。分离血清和血浆,测定血清白细胞介素 6(IL-6)和血浆酸性磷酸酶(ACP)水平,测大鼠脊柱和股骨骨密度。结果:与模型组相比,各中药组可显著降低 IL-6 和 ACP 水平($P < 0.05$ 或 $P < 0.01$),中药各剂量组及西药组间差异无显著性($P > 0.05$)。与模型组相比,各用药组骨密度均升高($P < 0.05$)。

主题词 骨质疏松/中医药疗法 骨密度/药物作用 @ 仙杞煎液

白细胞介素 6(IL-6)被认为是病理状态下调节骨吸收的重要因子。血浆酸性磷酸酶(ACP)水平反映破骨细胞活性和骨吸收状态。仙杞煎液是治疗本病的有效制剂,本实验旨在观察其对实验性骨质疏松大鼠血清 IL-6 和血浆 ACP 及其骨密度的影响。

1 材料和方法

1.1 实验材料:选用同济医科大学实验动物中心提供的 SD 雌性大鼠 72 只,7 月龄,体重 265~340g。仙杞煎液由猪卵巢、枸杞子、淫羊藿、山茱萸、丹参、牡蛎等药组成。猪卵巢取自 30~40 天龄雌性幼猪被阉割的卵巢,卵巢取出后清水漂洗,去除筋膜,置 80℃ 的烤箱内焙干后研细末入煎剂。其余药物常规煎煮提取上清液,离心去杂,4℃ 冰箱保存待用。终末浓度:低剂量组 0.3g/ml,中剂量组 0.6g/ml,高剂量组 1.2g/ml。尼尔雌醇片由北京四环制药二厂生产,京卫药准字(1996)第 153001 号。批号 200010801,1mg/片。仪器与试剂:美国 Norland 双能 X 线骨密度检测仪,由汉口铁路医院提供。XD811 生化分析仪由上海迅达仪器公司生产,湖北中医学院附属医院肝病中心提供。IL-6 定量 EIA 试剂盒由上海森雄科技实业有限公司提供,批号

20020103。ACP 试剂盒由南京建成生物工程研究所提供,批号 20020108。

1.2 实验方法

动物模型制备及评价标准:72 只 7 月龄 SD 雌性大鼠随机分为 2 组,手术组 60 只,假手术组 12 只。所有大鼠均用 2%戊巴比妥钠(25mg/kg)腹腔麻醉,手术组行双侧卵巢摘除术,假手术组于开腹后行伪手术,仅切除一段肠系膜,术后抗炎 3 天。手术组去卵巢术后 5 天开始连续 5 天阴道涂片,以证明去势彻底。去卵巢术后 60 天,两组各取 6 只,2%戊巴比妥钠(25mg/kg)麻醉后,用骨密度仪检测脊柱和右侧股骨骨密度。

分组给药:检查完毕,经 SPSS 软件行 t 检验统计有差异后,将手术组随机分为 5 组,即模型组、西药组、仙杞煎液剂量组、中剂量组、高剂量组,连同假手术组共 6 组。仙杞煎液低、中、高剂量组每天分别灌胃给药 1.6g/kg、3.2g/kg、6.4g/kg。西药组灌服尼尔雌醇液,尼尔雌醇按人鼠体表面积比率折算为 0.09mg/kg,临用前研碎,用生理盐水配成 0.01mg/ml 的混悬液,每周 1 次,假手术组与模型组均用生理盐水灌胃,每天 1.6g/kg。

标本采集:用药 60 天后,摘眼球取血后将大鼠处死,血样分两份。一份加肝素抗凝,分离出血浆,立即检测 ACP,采用化学比色法测定;另一份不抗凝,取血清,-20℃ 保存待测 IL-6,采用双抗体 ABC-ELISA 法检测。

统计学方法:采用美国 SPSS 软件包进行组间方差分析,所得数据以 $\bar{x} \pm s$ 表示。

2 实验结果

2.1 去势 2 个月后大鼠骨密度的变化:表 1 示,手术组大鼠去卵巢后骨密度下降,股骨骨密度与假手术组相比差异有显著性($P < 0.05$),说明绝经后骨质疏松大鼠模型成立。

2.2 用药 2 个月后各组大鼠骨密度比较:表 2 示,模型组较假手术组骨密度显著下降($P < 0.01$),各用药组骨密度均升高($P < 0.05$ 或 $P < 0.01$)。各剂量组及与西药组间差异无显著性($P > 0.05$)。

2.3 各组大鼠 IL-6 和 ACP 的变化比较:表 3 示,模

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表 1 去势 2 个月后大鼠骨密度比较(g/cm², $\bar{x} \pm s$)

组 别	鼠数(只)	脊柱	股骨
手术组	6	0.138±0.009	0.135±0.003*
假手术组	6	0.142±0.076	0.152±0.018

* 与假手术组比较, $P < 0.05$

表 2 各组大鼠骨密度比较(g/cm², $\bar{x} \pm s$)

组 别	鼠数(只)	脊柱	股骨
假手术组	6	0.138±0.015	0.146±0.010
模型组	6	0.115±0.003▲	0.126±0.005▲
低剂量组	6	0.132±0.011**	0.146±0.012**
中剂量组	6	0.143±0.010**	0.150±0.010**
高剂量组	6	0.148±0.031*	0.160±0.025**
西药组	6	0.143±0.026*	0.158±0.022**

与模型组比较, * $P < 0.05$, ** $P < 0.01$; 与假手术组比较, ▲ $P < 0.01$

表 3 各组大鼠 IL-6 和 ACP 比较($\bar{x} \pm s$)

组 别	鼠数	IL-6 含量(pg/ml)	ACP 含量(金氏单位/100ml)
假手术组	8	56.86±16.48	1.589±0.355
模型组	8	82.46±16.74△△	2.183±0.458△
低剂量组	7	58.00±22.34*	1.737±0.36
中剂量组	8	52.16±18.27**	1.581±0.331**
高剂量组	9	50.20±17.51**	1.556±0.326**
西药组	8	50.25±20.06**	1.693±0.345*

与模型组比较, * $P < 0.05$, ** $P < 0.01$; 与假手术组比较, △ $P < 0.05$, △△ $P < 0.01$

型组 IL-6、ACP 水平明显高于假手术组, 差异有显著性($P < 0.05$ 或 $P < 0.01$); IL-6 含量低剂量组、中剂量组、高剂量组、西药组均低于模型组, 差异有显著性($P < 0.05$ 或 $P < 0.01$); 中药各剂量组与西药组差异无显著性($P > 0.05$)。ACP 含量中剂量组、高剂量组、西药组与模型组差异有显著性($P < 0.05$ 或 $P < 0.01$)。各用药组间差异无显著性。

3 讨论

本方使用猪卵巢入药, 亦取其“以脏补脏”之意; 用枸杞子、山茱萸、山药滋补阴精; 用淫羊藿补益肾阳, 寓“阳中求阴”之意, 则肾阴阳双补, 精血充足。牡蛎滋阴潜阳, 收敛固涩, 且直接补充钙质。丹参能养血活血, 生新血, 去瘀血, 使瘀阻之脉络畅通, 血液中的营养物质能正常地通过哈佛氏系统进入骨骼, 上药共用可使精血充足, 髓充骨壮。

骨密度常用于判断骨量丢失、评定骨质疏松性和对骨折危险性进行预测, 本研究对大鼠脊柱和股骨进行定量分析显示, 大鼠去势后 2 个月, 脊柱、股骨骨密度与假手术组相比均下降, 尤以股骨骨密度下降明显($P < 0.05$), 说明绝经后骨质疏松症大鼠模型已经确立, 从去势后 4 个月检测结果来看, 随着大鼠月龄增长, 骨密

度进一步下降, 脊柱、股骨骨密度与假手术组相比, 有显著性差异($P < 0.05$)。用药后脊柱、股骨骨密度明显上升, 与模型组有显著性差异($P < 0.05$ 或 $P < 0.01$), 且与尼尔雌醇无明显差异。说明仙杞煎液同尼尔雌醇一样可以提高骨密度, 可用于绝经后骨质疏松的治疗。

IL-6 被认为是病理状态下调节骨吸收的重要因子。IL-6 是由许多细胞产生的具有广泛生物活性的细胞因子, 成骨细胞、破骨细胞、骨基质细胞、单核巨噬细胞均可分泌 IL-6, 且这些细胞因子表面均有其受体的表达, IL-6 通过其受体的旁分泌和自分泌作用于成骨细胞骨髓基质细胞, 从而刺激巨噬细胞集落形成单位即破骨细胞前体的形成。长期人类骨髓培养已证实, IL-6 能刺激多核细胞对破骨细胞表现型的表达, 在体外, IL-6 协同白细胞介素 1(IL-1)刺激破骨细胞聚集, 并使已被 ⁴⁵Ca 标记的大鼠释放 ⁴⁵Ca 和诱发骨质的吸收, IL-6 还刺激多发性骨髓瘤细胞的生长和局部溶骨^[1~3]。

ACP 主要存在骨、前列腺、血小板中, 血清 ACP 活性高于血浆是因为在凝血过程中血小板内 ACP 释放入血所致。采用雌性大鼠血浆, 排除了前列腺、血小板内 ACP 的影响, 此时血浆 ACP 主要来源于骨。骨性 ACP 由破骨细胞释放, 在骨吸收时对骨基质的溶解起重要作用, 当骨吸收活跃时, 反映为血中酶活性升高。血中的 ACP 水平反映破骨细胞活性和骨吸收状态。

本研究显示, 手术组 IL-6、ACP 与假手术组相比明显增加($P < 0.01$), 说明造模后骨吸收增加。仙杞煎液各剂量组均可降低 IL-6 和 ACP 水平, 与模型组差异有显著性($P < 0.01$), 与西药组差异无显著性($P > 0.05$)。说明中药仙杞煎液可以达到与尼尔雌醇相同的抑制骨吸收的效果, 王宝利认为淫羊藿可以抑制 IL-6 mRNA 的表达^[4], 而 IL-6 在骨微环境中, 由 IL-1、TNF 等刺激成骨细胞和骨基质细胞产生, 因此可以认为仙杞煎液可以直接作用于骨髓微环境, 发挥抑制 IL-6 等骨吸收促进因子的作用。

参考文献

- 1 Qu Q, Perala-Heape M, Kapanen A, et al. Estrogen enhances differentiation of osteoblasts in mouse bone marrow culture. Bone, 1998, 22(3): 201-209.
- 2 权金星, 李茂欣. 绝经后骨质疏松症的分子机理研究. 中华妇产科杂志, 2000, 35(12): 754-754.
- 3 谭春华, 周跃. 白细胞介素-6 与有关骨疾病的研究进展. 国外医学创伤与外科基本问题分册, 2000, 21(2): 77-79.
- 4 王宝利, 权金星, 郭善一, 等. 淫羊藿对去卵巢大鼠骨髓组织白细胞介素 6 mRNA 表达的影响. 中华妇产科杂志, 2000, 35(12): 724-724.

ABSTRACTS FROM ORIGINAL ARTICLES

Clinical observation on 102 cases of alcoholic fatty liver treated with Juge Yigan Decoction

Jia Jingpeng, Jiang Sen

Objective: To explore the ideal method to treat alcoholic fatty liver. **Methods:** 102 cases of alcoholic fatty liver were treated with Juge Yigan Decoction as treatment group, and the effect was compared with control group. **Results:** Effect of treatment group was superior to that of control group. Clinically cured rate, effective rate and ineffective rate in treatment group were 74.5%, 21.6% and 3.9% respectively, superior to 31.6%, 56.1% and 12.3% in control group. There was significant difference between two groups ($P < 0.01$). **Conclusion:** Juge Yigan Decoction is an ideal choice for treatment of alcoholic fatty liver.

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Key Words: Fatty liver, alcoholic/TCM therapy

(Original article on page 750)

Clinical observation on 123 cases of rheumatoid arthritis treated with Sitengyin

Xu Feng, Dai Xiaoming, Zhang Guanghua, et al.

Objective: To observe the effect of Sitengyin on rheumatoid arthritis (RA). **Methods:** 123 cases of RA were treated with Sitengyin, and 67 cases were given Methotrexate and Nimesulide as control. Main symptoms, signs and results of related laboratory tests were compared between two groups before and after treatment. **Results:** Markedly effective rate and total effective rate of treatment group were 72.36% and 95.94% respectively, and those of control group were 65.67% and 86.57%. There was significant difference in efficacy between the two groups ($P < 0.05$). Compared with control group, improvement of main symptoms, signs and results of laboratory tests of treatment group was more remarkable with less side-effect, indicating that efficacy of treatment group was superior to that of control group. **Conclusion:** Sitengyin is definitely effective in treatment of RA, with less side-effect. It is of great value of application and development.

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Key Words: Arthritis, rheumatoid/TCM therapy

(Original article on page 760)

Experimental study on fluorescence histochemistry and ultra-microstructure changes of cardiac muscle of model rats with heart deficiency syndrome

Liu Liqing, Zhou Shengnian

Objective: To observe the effect of heart deficiency syndrome on microstructure and ultra-microstructure of cardiac muscle cells.

Methods: Healthy rats were randomly divided into model group and control group. For model group, cardiac muscle was taken respectively after 48 hours and 72 hours to make frozen slices and ultra-filmy slices. Fluorescence and electric microscope were used to observe changes of distribution, content and ultra-microstructure of heparin and DNA within cardiac muscle cells. **Results:** After 48 hours, fluorescence PAS reaction of cardiac muscle cells of model group reduced significantly, and after 72 hours, electropositive matters in DAN fluorescence reaction was much less than that of control group. Based on tridimensional and quantificational analysis, there was significant difference in chondriosome density between two groups ($P < 0.01$). **Conclusion:** Syndrome of heart deficiency could result in injury of cardiac muscle cells and affect metabolism of cardiac muscle and structure of cardiac muscle cells.

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Key Words: Heart deficiency syndrome/pathology, cardiac muscle/ultra-microstructure

(Original article on page 775)

Effect of Xianqi Decoction on bone mass density, IL-6 and plasma acid phosphatase in experimental osteoporosis rats

Zhang Lijun, Zhang Yan, Xu Qian, et al.

Objective: To investigate the effect of Xianqi Decoction on experimental osteoporosis of rats and its mechanism. **Methods:** 72 female SD rats aged 7 months were randomly divided into two groups, i. e. 60 for operation group, 12 for sham-operation group. Bilateral ovariectomy was performed on operation group. 2 months later, postmenopausal osteoporosis model rats were successfully established. Rats in operation group were randomly divided into model group, western medicine group, Xianqi Decoction low dose group, middle dose group and high dose group. Total six groups were given corresponding medicine by intragastric perfusion. 60 days later, rats were sacrificed after taking blood sample. Serum IL-6, plasma acid phosphatase (ACP) and bone mass density of vertebra and femur were determined. **Results:** Compared with model group, IL-6 and ACP level in three Xianqi Decoction groups was significantly reduced ($P < 0.05$ or $P < 0.01$), and there was no significant difference among Xianqi Decoction groups and western medicine group. Bone mass density in all drug-administered groups was increased ($P < 0.05$).

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Key Words: Osteoporosis/TCM therapy, Bone mass density/drug effect

(Original article on page 780)