

补肾方药对地塞米松诱发的骨质疏松大鼠体内雌激素和 1,25 羟维生素 D₃ 的影响

河北医学院生化教研室(石家庄 050017) 刘和娣 李 恩 佟晓旭

内容提要 本实验应用地塞米松(0.1 mg/100 g 体重, 2 次/周, 连续 5 周), 同时给予低钙饲料, 诱发大鼠发生骨质疏松, 观察血中雌二醇(E₂), 1,25 羟维生素 D₃ [1,25-(OH)₂D₃] 的变化, 并给予钙剂、中药治疗, 以研究 E₂、1,25-(OH)₂D₃ 和骨质疏松发生的关系及相互影响。结果提示: 骨质疏松大鼠体内 E₂、1,25-(OH)₂D₃ 水平均较低($P < 0.01$ 、 0.05)。通过补肾方药或钙剂治疗后, 骨质疏松明显改善, E₂、1,25-(OH)₂D₃ 得到回升, 提示 E₂、1,25-(OH)₂D₃ 的降低和骨质疏松密切相关, 可能首发 E₂ 降低, 继发 1,25-(OH)₂D₃ 降低。中医补肾方药能影响 E₂、1,25-(OH)₂D₃ 的代谢, 治疗骨质疏松。

关键词 骨质疏松 雌二醇 钙剂 1,25 羟维生素 D₃

老年骨质疏松已成为严重威胁人类健康问题, 其发病机理尚不清楚。近代研究将骨质疏松分为两类^[1,2], 绝经后骨质疏松(I 型)和老年性骨质疏松(II 型)。前者与雌激素降低有关^[2]; 后者机理, 大多数学者倾向于^[2,3], 随着年龄增长, 血清 1,25 羟维生素 D₃ [1,25-(OH)₂D₃] 水平下降, 肠钙吸收减少, 尿钙排泄增加, 负钙平衡, 继发甲状旁腺素(PTH)升高, 溶骨增强, 渐至骨质疏松。本实验旨在通过动物实验阐述 E₂、1,25-(OH)₂D₃ 与骨质疏松发病的关系及两者之间的相互影响。

材料与方法

参照天津药检所张履祥等建立大鼠骨质疏松的实验方法制作动物模型^[4], 实验动物为本校动物室提供的纯系 Wistar 大鼠, 饲料为本校动物室配制, 低钙饲料含钙量为 0.2%, 正常饲料与高钙饲料均是在低钙饲料的基础上加碳酸钙调整, 其钙含量分别是 0.6%、1.2%, 地塞米松为市售针剂(2 mg/ml), 用前生理盐水稀释 4 倍, 以便用量准确, 中药经我校生药组鉴定系正品。

实验选用 4 月龄健康雌性大鼠 44 只, 体重平均 290 g 左右, 常规喂养 1 周后, 其中随机取 33 只肌肉注射地塞米松(0.1 mg/100 g 体重, 2 次/周), 配合食用低钙饲料, 饮用蒸馏水, 诱发大鼠骨质疏松作为病理模型。其余 11 只食用正常饲料, 肌肉注射生理盐水, 作为正常对照组。

5 周后, 随机处死模型组 3 只、正常组 1 只, 取

第 1 腰椎做切片, 发现地塞米松组骨小梁减少, 骨组织结构紊乱。同时随机取正常组及病理组各 10 只大鼠心脏取血, 测血中 E₂、1,25-(OH)₂D₃ 含量。随后把病理模型大鼠在上述喂养条件下分为 3 组, 即把其中 10 只改低钙饲料为高钙饲料喂养, 为高钙治疗组; 10 只加喂中药浓缩液(0.5 ml/100 g 体重, 此量根据人体用量折合而成), 为中药治疗组; 其余 10 只为病理对照组。分别在中药、钙剂治疗 7 周、11 周后, 心脏取血, 测血中 E₂、1,25-(OH)₂D₃ 含量, 并于最后一次取血后处死动物, 取第一腰椎做骨切片, 以观察组织形态学的改变。

根据补肾组方选用熟地、山药、仙灵脾等, 常规煎煮, 浓缩后灌胃, 每日 1 次, 浓缩液生药含量 1.6 g/ml。

E₂ 放免测定药盒由北方免疫试剂研究所提供, 按其说明书操作; 1,25-(OH)₂D₃ 采用放射性蛋白结合法测定, 由白求恩医科大学中心实验室同位素组协助完成。

结 果

一、血中 E₂ 含量的变化 见附表。测定结果表明, 地塞米松应用 5 周后, 与正常组比较雌激素水平明显降低($P < 0.01$)。3 组仍继续用地塞米松, 其中中药组和高钙组治疗 7 周后, 两治疗组雌激素水平得到回升, 与正常组比较无统计学差异, 而病理组雌激素水平进一步降低, 与正常组、中药组、高钙组比较均有显著性差异($P < 0.001$ 、 0.02 、 0.05)。中药和

钙剂治疗 11 周后第 3 次取血的结果与此相似, 两次之间纵向比较无统计学差异。结合组织学形态观察发现, E_2 的降低和骨质疏松的发生密切相关, 中药和钙剂治疗 7 周便能影响 E_2 的水平, 对骨质疏松有一定治疗作用。

二、血中 $1, 25-(OH)_2D_3$ 含量的变化 见附表。治疗前, 病理组血 $1, 25-(OH)_2D_3$ 含量下降, 与正常组比较有显著差异 ($P < 0.05$)。治疗 7 周取血测得中药、钙剂治疗组 $1, 25-(OH)_2D_3$ 的水平得到回升, 与病理组比较有显著差异 ($P < 0.05$), 与正常组比较无

统计学差异。治疗 11 周取血分析结果与此类似, 结合组织学形态观察, 提示 $1, 25-(OH)_2D_3$ 的水平降低与骨质疏松的发生密切相关, 且与 E_2 水平成正相关 ($r = 0.4055$, $n = 200$, $P < 0.001$)。

三、组织学形态的改变 可见病理组骨小梁数目明显减少, 排列不整, 骨髓量增加, 高倍视野见成骨细胞减少, 破骨细胞增多, 而中药组和钙治疗组和正常组比较变化不明显, 说明中药和钙剂对骨质疏松均有一定治疗作用。

附表 各组治疗前后 E_2 、 $1, 25-(OH)_2D_3$ 含量比较 (pg/ml, $\bar{x} \pm S$)

组别	动物数	治疗前		治疗 7 周		治疗 11 周	
		$1, 25-(OH)_2D_3$	E_2	$1, 25-(OH)_2D_3$	E_2	$1, 25-(OH)_2D_3$	E_2
病理	10	35.38 ± 17.06	90.36 ± 23.60	33.38 ± 16.00	81.46 ± 18.10	36.68 ± 13.63	87.99 ± 19.19
正常	10	$53.64 \pm 16.07^*$	$123.27 \pm 18.50^*$	$49.41 \pm 9.95^*$	$124.27 \pm 19.21^{**}$	$54.92 \pm 20.16^*$	$127.70 \pm 16.84^{**}$
高钙	10	—	—	$52.59 \pm 14.92^*$	$110.87 \pm 34.76^*$	$57.36 \pm 20.67^*$	$115.50 \pm 29.76^*$
中药	10	—	—	$50.35 \pm 15.41^*$	$115.49 \pm 28.32^*$	$54.80 \pm 17.46^*$	$121.44 \pm 26.27^*$

注: 各组与病理组比较, $^*P < 0.05$, $^{**}P < 0.001$

讨 论

实验结果表明: 大量的钙剂补给对实验性大鼠骨质疏松有一定防治作用。 E_2 、 $1, 25-(OH)_2D_3$ 、钙剂三联疗法用于骨质疏松的治疗有效^[5,6], 也间接证明 E_2 、 $1, 25-(OH)_2D_3$ 及钙代谢的失衡是骨质疏松发生的重要原因。

根据中医学肾主骨生髓的理论及老年人存在肾阴阳两虚的事实, 提示治疗骨质疏松可以从中医补肾入手。我室采用自拟方药对地塞米松诱发的骨质疏松大鼠进行实验治疗, 发现补肾疗法能改善大鼠骨质疏松状态, 使骨小梁增多、粗大, 且大鼠体内 E_2 、 $1, 25-(OH)_2D_3$ 的水平增高。资料表明^[7,8]: 在骨质疏松患者体内, 血中 $1, 25-(OH)_2D_3$ 水平是低的。有些学者认为^[9]: 随年龄增长, 肾脏排泄功能的下降伴随着肾脏 1α 羟化酶活性的降低。尚有人认为^[10]: 绝经后雌激素水平的降低, 对 1α 羟化酶的刺激作用减弱是 $25-OH-D_3$ 转化为 $1, 25-(OH)_2D_3$ 减少的重要原因。补肾可以提高体内雌激素水平的疗法已用于女性更年期综合征的治疗^[11]。本实验用补肾方药治疗大鼠骨质疏松, 大鼠体内雌激素水平升高, $1, 25-(OH)_2D_3$ 也相应升高, 且成正相关。提示补肾疗法通过对体内激素的调整, 使雌激素水平增加, 刺激 1α 羟化酶的活性, 使 $1, 25-(OH)_2D_3$ 增高, 促使钙结合蛋白合成, 利于肠钙吸收, 改善骨骼状况。

参 考 文 献

1. Riggs B, et al. Evidence for two distinct syndromes of

involution osteoporosis. Am J Med 1983; 75:889.

2. Riggs B, et al. Involutional osteoporosis. N Engl J Med 1986; 314: 1676.

3. Orwoll E, et al. Alterations in calcium, vitamin D and parathyroid hormone physiology in normal men with aging: Relationship to the development of senile osteoporosis. J Clin Endocrinol Metab 1986; 63: 1262.

4. 张履祥, 等. 复方钙剂对皮质激素引起大鼠骨质疏松的实验治疗. 天津医药 1985; 11: 670.

5. Jensen GF, et al. Treatment of postmenopausal osteoporosis: A controlled therapeutic trial comparing oestrogen/gestagen, $1, 25-(OH)_2D_3$ and calcium. Clin Endocrinol 1982; 16: 515.

6. Tilyard M. Low dose calcitriol versus calcim in established postmenopausal osteoporosis. Metablism 1990; 39 (4, suppl 1): 50.

7. Gallagher JC, et al. Action of $1, 25-(OH)_2D_3$ on calcium balance and bone turnover and its effect on vertebral fracture rate. Metablism 1990; 39 (4, suppl 1): 30.

8. Deluca HF. Osteoporosis and the metabolism of vitamin D. Metablism 1990; 39 (4, suppl 1): 3.

9. Tsai KS, et al. Impaired vitamin D metabolism with aging in women possible role in pathogenesis of senile osteoporosis. J Clin Invest 1984; 73: 1668.

10. Ettinger B, et al. Postmenopausal bone loss is prevented by treatment with low dosage estrogen and calcium. Am Intern Med 1987; 106: 40.

11. 范振远, 等. 六味地黄丸(汤)药理研究及临床应用近况. 中成药 1988; 12: 35.

Effects of Replenishing Kidney Herbs on Estrogen and 1,25-(OH)₂-D₃ of Dexamethasone-induced Rats Model with Osteoporosis

Liu He-di (刘和娣), Li En (李恩), Tong Xiao-xu (佟晓旭)

Dept. of Biochemistry, Hebei Medical College, Shijiazhuang (050017)

Estrogen and 1,25-(OH)₂-D₃ change in rats with osteoporosis induced by dexamethasone (DXM) was observed. The calcium and replenishing Kidney Herbs (RKH) was used in treating osteoporosis. The results shown: The level of estrogen and 1,25-(OH)₂-D₃ in rats with osteoporosis was lower than that of the normal. Calcium and RKH were effective in treating osteoporosis and they could recover the level of estrogen and 1,25-(OH)₂-D₃ to normal state, the effect of RKH was better than that of calcium. There was positive correlation between the level of estrogen and 1,25-(OH)₂-D₃. The treatment of RKH might be an optimal method for osteoporotic treatment.

Key word osteoporosis, calcium, estrogen, 1,25-(OH)₂-D₃

(Original article on page 544)

Ultrastructural Observation of Intratumoral Neutrophils and Macrophages Induced by Garlic Oil

Gao Yu-min (高玉民), Xie Jin-yu (谢锦玉), Piao Ying-jie (朴英杰)

General Hospital of Air Force, PLA, Beijing (100036)

After injection of garlic oil in tumor focus a large amount of neutrophils, macrophages and lymphocytes appeared. Some neutrophils and macrophages located adjacent to the tumor cells, some processes of neutrophils and macrophages penetrated into intracellular body of tumor cells. This result showed that garlic oil could induce neutrophils and macrophages against tumor.

Key word garlic oil, neutrophils, macrophages, antitumor

(Original article on page 546)

Effect of Glycerol-Induced Acute Renal Failure in Rabbit with *Ligusticum Wallichii* on TXB₂, 6-keto-PGF_{1α} and 6-keto-PGF_{1α}/TXB₂

Hu Wei-lie (胡卫列), Ma Yong-jiang (马永江), et al

Dept. of Urology, Guangzhou General Hospital of PLA, Guangzhou (510010)

The effect in preventing and treating glycerol-induced acute renal failure (ARF) in rabbit with *Ligusticum wallichii* (LW) has been studied. 33 male and female rabbits weighing 2.0–3.0 Kg were divided into three groups randomly: (1) LW treated group. (2) pathological control group and (3) normal control group. The measurement of plasma 6-keto-PGF_{1α}, TXB₂ concentration and 6-keto-PGF_{1α}/TXB₂ ratio were carried out with radioimmunoassay after 24 hr, 48 hr and 72 hr of ARF. The results showed that plasma TXB₂ concentration obviously increased ($P < 0.01$), 6-keto-PGF_{1α} concentration had no obvious changes ($P > 0.05$), 6-keto-PGF_{1α}/TXB₂ ratio markedly decreased and LW could reduce plasma TXB₂ concentration, slightly increase the plasma 6-keto-PGF_{1α} concentration, keep 6-keto-PGF_{1α}/TXB₂ ratio in normal level after ARF. It showed that LW could inhibit effectively platelet activation, correct 6-keto-PGF_{1α}/TXB₂ imbalance and have a preventing and treating effect for ARF.

Key word *Ligusticum wallichii*, acute renal failure, TXB₂, 6-keto-PGF_{1α}

(Original article on page 549)