

实验研究

“骨宝丸”防治佝偻病的实验研究

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摘要 本文观察了佝偻病大鼠在用中药“骨宝丸”治疗前后血清钙、磷、碱性磷酸酶、25 羟基维生素 D₃ 及骨组织形态学和骨计量学的变化,并与维生素 D₃ 进行了比较。结果表明:“骨宝丸”防治佝偻病有效,特别是在提高血钙、血磷和降低血碱性磷酸酶以及促进类骨质矿化方面效果显著,但其作用机理与维生素 D₃ 不同。

关键词 佝偻病 骨宝丸 预防与治疗

本文以佝偻病大鼠为模型,观察了中药“骨宝丸”对佝偻病的防治作用,并对其作用机理进行初步探讨。

材料和方法

1. 实验药物:“骨宝丸”,由佛山市中医院提供。维生素 D₃ 灭菌油溶液。

2. 实验动物及分组:将 Wistar 雄性大鼠分为 5 组:(1)佝偻病组;(2)佝偻病低剂量“骨宝丸”组(0.175g/ml. day, po. 共 30 天);(3)佝偻病高剂量“骨宝丸”组(0.35g/ml. day, po. 共 30 天);(4)佝偻病维生素 D₃ 组(与其他组同时起步,一次肌肉注射维生素 D₃ 0.24 万 IU/只)上述四组动物均采用 VitD 缺乏饮食及避光饲养;(5)正常对照组(不避光,常规饲养)。第 31 天,分别取血做生化测定;取股骨远端及胫骨近端做骨形态学和骨计量学测定。

3. 实验方法:

(1)血清生化:1)血清钙(Ca):EDTA 滴定法。2)血清磷(P):抗坏血酸还原法。3)血碱性磷酸酶(ALP):硝基酚磷酸钠法。4)25 羟基维生素 D₃ [25(OH)D₃]:采用竞争性蛋白结合法测定^[1] 25 羟基 [26, 27-甲基-³H] 维生素 D₃ 购自英国放化中心,25(OH)D₃ 标准品为 F-HOFFMAN La ROCHE 产品。本测定批内、批间差异分别为 9.1% 和 10.8%,平均回收率为

90.0±8.7%。

(2)骨组织及骨计量学测定:取各组大鼠一侧股骨远端及胫骨近端,用 10% 福尔马林固定,10% EDTA 脱钙,制备石蜡切片,HE 染色,光镜观察干骺端骨组织结构。

部分动物用盐酸四环素标记,剂量为 40ml/kg,采用二次标记法,于第二次注射后二天,将动物经腹主动脉放血处死,取另侧股骨标本置 70% 乙醇固定,然后用 0.5% Villanueva 染色液染色 72 小时,依次用 95%、100% 乙醇和丙酮脱水脱脂,再用甲基丙烯酸甲脂包埋,制成不脱钙骨切片,用 Olympus 荧光显微镜,在波长 360nm 蓝色激发荧光下观察^[2]。

各种切片均用测微尺或网格测微计进行有关计量测定。

实验结果

1. 血清生化的改变:

未给药的佝偻病大鼠血清 ALP 明显高于正常对照组(P<0.001),25(OH)D₃ 明显低于正常对照组(P<0.001)。灌服“骨宝丸”后,血清 ALP 明显下降,与佝偻病组相比较(P<0.001),但 25(OH)D₃ 含量未见回升。维生素 D₃ 组恰相反:ALP 未见改变,25(OH)D₃ 明显回升,以至明显高于正常对照组(P<0.001)。佝偻病组血清 Ca、P 无明显变化,但 Ca、

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P 乘积高于正常对照组。用药后,各组 Ca、P 水平多有明显升高(除高剂量“骨宝丸”组外),Ca、P 乘积全部显著高于佝偻病组 ($P < 0.001$)。

2. 骨组织的改变及骨计量测定:

(1)不脱钙骨切片观察:正常对照组:骨皮质内哈佛氏管数目减少,管腔小,类骨质较少,平均类骨质缝宽度[mean osteoid seam width, (MOSW)]为 $1.681\mu\text{M}$ 。佝偻病组:骨皮质内哈佛氏管增大,数目增多,类骨质覆盖骨小梁的比例增加,宽度也增加,四环素荧光标记面增多。MOSW 为 $4.227\mu\text{M}$ 。为正常对照组的 2.5 倍。

“骨宝丸”低剂量组:哈佛氏管数量减少,管腔变小,四环素荧光标记面增多,MOSW 为 $2.693\mu\text{M}$,比佝偻病组下降 36.3%。

“骨宝丸”高剂量组:哈佛氏管数量少于低剂量组,管腔变小,但荧光标记仍较多,MOSW 为 $1.824\mu\text{M}$,比佝偻病组下降 56.8%,接近正常对照组。

维生素 D₃ 组:哈佛氏管数量减少,管腔变小,四环素荧光标记的佝偻病组明显减少。MOSW 为 $1.069\mu\text{M}$,比佝偻病组下降 74.7%。

(2)脱钙骨切片观察:佝偻病组大鼠股骨远端骺板总厚度、增生层及肥大细胞层厚度与其他各组相比为最厚,而“骨宝丸”高剂量组及维生素 D₃ 组上述各层厚度明显减少 ($P < 0.05$)或有减少趋势。“骨宝丸”低剂量组上述各层厚度虽有减少趋势,但作用不及其他两组明显。胫骨近端骺板各层厚度变化与股骨远端骺板的变化相似。

讨 论

“骨宝丸”是佛山市中医院著名骨科专家陈渭良先生根据其多年使用的经验方制成的小蜜丸,主要成份有龟板胶、杜仲、仙茅、山萸肉、枸杞子、黄芪、红参、淮山药等。主治肝肾亏损诸证。

本实验证实“骨宝丸”对大鼠佝偻病有防治作用。血生化测定表明:该药具有升高佝偻病大鼠血清钙、磷和钙磷乘积的作用,并可显

著降低血 ALP 的含量。骨组织形态学及计量测定表明:“骨宝丸”可明显减少佝偻病大鼠骺板总厚度和增生层、肥大细胞层厚度,可明显减少类骨质缝宽度。同时,可减少佝偻病大鼠骨皮质内哈佛氏管数目,缩小哈佛氏管管腔,减少类骨质覆盖小梁的比例等。上述观察结果说明“骨宝丸”促进了类骨质的矿化。

维生素 D₃ 同样具有促矿化作用,但作用机理与“骨宝丸”不同。维生素 D₃ 组测定结果显示血清 25(OH)D₃ 含量明显增多。这是源于对佝偻病大鼠提供了足够的维生素 D₃,从而产生其代谢产物 25(OH)D₃ 和 1,25(OH)₂D₃,以增加了肾、小肠对 Ca、P 的重吸收,有利于骨基质的矿化。维生素 D₃ 组血清 Ca、p 的增加与 25(OH)D₃ 的增加是相符的,但它没有改变佝偻病大鼠血 ALP 增高的现象。“骨宝丸”的作用显然不是由于增加了血清 25(OH)D₃ 含量的结果。因为,服用“骨宝丸”后没有改变佝偻病大鼠血清 25(OH)D₃ 含量低下的状态。该药增加血清 Ca、P,促进骨质矿化的机制可能是:(1)直接提供矿化元素。“骨宝丸”主要成份含有较丰富的钙、镁、锌、铁等微量元素,直接有助于类骨质的矿化;(2)促进骨细胞的生长发育,从而调节骨基质的矿化。“骨宝丸”组动物血清 ALP 含量明显低于佝偻病组,而接近于正常对照组的水平。这表明“骨宝丸”对骨代谢具有保护作用。一般认为骨的严重损伤或骨疾病(例如:佝偻病,软骨病、成骨肉瘤、骨折愈合期、甲状旁腺功能亢进等)都有不同程度的血清 ALP 含量增高,这是由于成骨细胞含有高浓度的 ALP 释放入血中的缘故。该药物使血清 ALP 含量降低,说明了对骨细胞生长发育的良好调节作用。

“骨宝丸”的作用是否还与甲状旁腺激素相关,尚有待进一步探讨。

参考文献

1. 薛延,等. 25 羟基维生素 D 浓度的竞争性蛋白结合测定法. 创伤骨科学报 1990;2:103.
2. 邱明才,等. 正常人骨动力学的初步研究. 天津医药 1987;5:267.

Abstract of Original Articles

Experimental study on Gu Bao Wan in treating rachitis

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In this article, changes of serum calcium, phosphorus, alkaline phosphatase, 25-hydroxy-vitamin D₃, bone tissue morphology and bone metrology were observed in treating rachitic rats with Chinese medicine Gu Bao Wan pre- and post-treatment and they were compared with Vitamin D₃. The results indicated that Gu Bao Wan is effective in the prevention and treatment of rachitis, especially it has prominent effects in the elevation of serum calcium, phosphorus, and decreasing of serum alkaline phosphatase and in the promotion of mineralization of os- teoid. But it is different from the mechanism of Vitamin D₃.

Key Words Rachitis Gu Bao Wan Prevention and treatment

(Original article on page 5)

Laboratory study of rotatory manipulation in the treatment of lumbar intervertebral disc protrusion

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The stress changes on posterior lateral edges of L_{4-5} , $L_5 - S_1$ discs and the positional alter- nates of articular processes of lumbar facet joints were measured while mimicing rotatory manip- ulations were performed on three spinal specimens from fresh cadavers. The study showed that rotatory manipulation with anterior and lateral flexion allowed a greater range of motion be- tween articular processes of lumbar facet joints than straight rotatory manipulation. There was a sliding movement between articular processes while the spine was rotated. The interarticular space of the right facet joint was increased when the spine rotated to the left and vice versa. The sliding movement between the articular processes can adjust the position of lumbar vertebra. The pressure was increased at the left posterior lateral site of the disc and was decreased at the right posterior lateral site of the disc while the spinal specimen was rotaed during flexion to the left, and vice versa. Negative pressure would be changed to positive at the end stage of rotatory manipulation. Such kind of repeated changes of pressure will change the position and shape of the protruded nucleus, and modification of the pressure on the nerve root would be happened.

Key Words Lumbar intervertebral disc protrusion Manipulation therapy Biomchanics

(Original article on page 7)

Experimental study on the influence of anti-bending force of the femur of senile rats with Fu Fang Wu Ming Yi Chong Ji

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Thirty-two twelve months old senile rats were divided randomly into four groups with eight in each group. Subjects in the experimental group were feeded with food and Fu Fang Wu Ming Yi Chong Ji (pyrolusite diluent, Chong Ji). The subjects in control group were feeded with food only. All of them were sacrificed at 18-month old. Bending destroyed load and thickness of femoral cortex were measured. The results indicated that the bending destroyed load of both sex of the rats and average cortex thickness were prominently higher than the control group ($p < 0.05 - 0.01$). In both experimental and control group, the bending destroyed load of male rats was prominently higher than that of female ones ($p < 0.01$). This indicates that Chong Ji bears the action of prevention and delaying onset and developing of osteoporosis. In the same age group, bone loss of female rats are relatively evidently than that of male ones.

Key Words Osteoporosis Wu Ming Yi Chong Ji Biomechanics

(Original article on page 10)

Integration of traditional Chinese and modern medicine and intramedullary treatment of fracture of femoral shaft

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Three hundred and sixty five cases of fracture of femoral shaft were treated with integration of traditional Chinese and modern medicine. A comparative analysis and exploration of these two methods were carried on in order to better application in the treatment of fracture of femoral shaft.

Key Words Fracture of femoral shaft Traditional Chinese medicinal therapy

Fixation of fracture, intramedullary

(Original article on page 12)

Enhanced clamp fixator in the treatment of fracture of olecranon

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Two hundred and ten cases of fracture of olecranon were treated with enhanced clamp fixator. There were a rate of anatomical or near anatomical reduction of 97.6% after a follow-up period from three months to eight years. It is realized that the instrument can be used in any type of fracture of olecranon, the key point is to select suitable fixating point based on different types of fracture.

Key Words Fracture of olecranon Enhanced clamp fixator Fracture fixator

(Original article on page 21)