

活骨冲剂治疗股骨头无菌性坏死的实验研究

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内容提要 本研究是在活骨冲剂治疗股骨头无菌性坏死临床观察基础上进行其药效学及急性毒性试验。结果表明: (1)本品有明显的抗炎镇痛作用,并有促进血清 Ca、P 吸收及促进鸡胚股骨生长等补肾壮骨作用。(2)急性毒性试验过程中未测出 LD₅₀; 又进行最大耐量测定, 24 h 内总给药量为 50 g/kg(相当于成人 1 次临床用药量的 166.7 倍), 亦未测出 LD₅₀。

关键词 活骨冲剂 股骨头无菌性坏死 药效学实验

近年来, 应用中药活骨冲剂治疗股骨头无菌性坏死的临床观察中确实有较好疗效。本研究是在此基础上, 进行了药效学及急性毒性试验, 为临床治疗提供了理论依据。

实验材料

一、药物 活骨冲剂由肉桂、骨碎补、川断、熟地、山萸肉、鹿角胶、黄芪、白芍、血竭、龙骨、牡蛎等中药组成。先制成浸膏, 再加白砂糖, 经干燥后制成颗粒, 每袋 15 g(含生药 10 g), 供临床使用。本实验样品用浸膏加水使其完全溶解, 制成溶液, 最大浓度为 50%。均为本院制剂室提供, 收试批号 920728。醋酸氢化可的松由哈尔滨制药厂生产, 批号 910728; 阿斯匹林由北京制药厂生产, 批号 911020; 龙牡壮骨冲剂为市售。冰乙酸和二甲苯为黑龙江省中医研究院病理室提供。

二、实验动物 昆明种小白鼠, 雌雄兼用, 体重 18~22 g/只, 由哈尔滨铁路中心医院动物室提供。

方法与结果

一、抗炎镇痛试验

1. 活骨冲剂对小鼠耳部炎症及毛细血管通透性的影响, 按文献⁽¹⁾。选健康小鼠 50 只, 随机分为 5 组, 每组 10 只, 即生理盐水对照组, 给等量生理盐水灌胃; 氢化可的松阳性对照组(简称氢可组)按 20 mg/kg 剂量腹腔注射氢化可的松; 活骨冲剂小、中、大剂量组按 1.8 g/kg(成人日治疗量的 2 倍, 以此类推)、3.6 g/kg 及 4.5 g/kg, 每日 1 次灌胃, 连续 3

日, 于末次给药 40 min 后, 每只小鼠左耳均匀涂布二甲苯 0.03 ml 致炎, 15 min 后断颈处死小鼠, 以打孔器将小鼠双耳间部位等面积切下称重, 用左右耳片重量差表示肿胀度, 求其肿胀抑制率。结果表明: 氢化可的松可显著减轻炎症反应, 活骨冲剂亦具有同样效果, 中、大剂量组与盐水对照组比较有显著性差异, $P < 0.05$ (表 1)。

表 1 活骨冲剂对小鼠抗炎、血管通透性及镇痛作用的影响 ($\bar{x} \pm S$)

组别	鼠数 (只)	耳廓肿胀 (mg)	吸收度 (OD 值)	扭体次数 (次)
盐水对照	10	7.36±1.12	0.084±0.045	36.5±3.7
氢可	10	4.81±1.02*	0.041±0.038**	12.8±1.9***
活骨冲剂				
4.5 g/kg	10	4.91±1.13*	0.050±0.022**	18.0±2.1**
3.6 g/kg	10	5.01±1.03*	0.062±0.013*	23.8±4.1*
1.8 g/kg	10	5.28±1.31	0.072±0.051	27.1±2.8

注: 与对照组比, * $P < 0.05$, ** $P < 0.01$; ▲阿斯匹林

观察活骨冲剂对小鼠毛细血管通透性的影响, 取健康小鼠 50 只, 分组、给药方法、途径同上。给药 60 min 后给 0.5% Evans 蓝溶液 5 ml/kg 静脉注射, 5 min 后予 0.7% 醋酸 0.1 ml/10 g 腹腔注射, 30 min 后处死, 以蒸馏水反复冲洗腹腔内 Evans 蓝溶液至 10 ml, 加 0.1 mol/L NaOH 0.1 ml 后用 721 分光光度计比色(590 nm), 结果见表 1。表 1 示, 中、大剂量活骨冲剂对醋酸所致小鼠腹腔毛细血管通透性增强有显著抑制作用($P < 0.05$, $P < 0.01$), 结果表明活骨冲剂有显著抗炎作用。

2. 镇痛试验 扭体法, 按文献⁽²⁾。取健康小鼠 50 只, 分组、给药方法及途径同上, 仅氢可组改为阿

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斯匹林组(0.2 g/kg)。于第3天给药50 min后,予0.7%醋酸0.2 ml/只腹腔注射,记录给药15 min内扭体次数,结果阿斯匹林组、活骨冲剂中、大剂量组的小鼠扭体次数明显少于盐水对照组($P < 0.01$, $P < 0.05$, $P < 0.01$),表明活骨冲剂有显著镇痛作用。

二、对小鼠Ca、P吸收作用的影响,按文献⁽³⁾

取健康小鼠50只,分组、给药方法与剂量同上,仅阳性对照组给龙牡壮骨冲剂4.5 g/kg。每组均每日1次灌胃,连续10日。于末次给药后60 min,取血分别用“乙胺四乙酸二钠滴定法”与“硫酸亚铁磷钼蓝比色法”测定血清Ca、P,结果见表2。

表2 活骨冲剂对小鼠Ca、P含量的影响 ($\bar{x} \pm S$)

组别	鼠数 (只)	血清Ca (mmol/L)	血清P (mmol/L)
对 照	10	2.58±0.24	1.65±0.11
龙牡冲剂	10	3.03±0.31*	2.33±0.12*
活骨冲剂			
4.5 g/kg	10	2.98±0.30*	2.20±0.14*
3.6 g/kg	10	2.78±0.18	1.97±0.17
1.8 g/kg	10	2.42±0.21	1.55±0.15

注:与对照组比, * $P < 0.05$

表2可见,4.5 g/kg活骨冲剂、龙牡壮骨冲剂与盐水对照组比较,血清中Ca、P含量均显著升高(P 均 < 0.05)。

三、对促进试管内鸡胚股骨生长的作用,按文献⁽⁴⁾。从孵化9日的京白鸡卵中取鸡胚并置于盛有Hank's液的烧杯中,洗涤后放在培养皿中,将两下肢从髌髌关节处分离开,在不破坏股骨骨髓的情况下,制成完整的股骨标本。在每个鸡胚的一对股骨中,左侧为给药组,右侧为空白自身对照组;给药组试管中加入混合液2 ml(药液:培养液=2:100),后者试管中加入同体积培养液,加棉塞后,置入38℃的含5%CO₂的恒温培养箱中。培养管的倾斜度为5~10°,每6 h摇动1次,共4次。第3日更换培养液,至第7日取出,用生理盐水洗净,除去表面的水分,在解剖镜下用4分尺测量标本的长度。结果活骨冲剂组股骨长度为5.712±0.304 mm,空白对照组为5.404±0.246 mm;提示活骨冲剂有较好的促进鸡胚股骨生长作用,与对照组比较有显著性差异($P < 0.05$)。

四、急性毒性试验,按文献⁽⁵⁾。

1. 半数致死量(LD₅₀)测定:选健康小鼠50只,随机分为5组,每组10只,按10.2、12.8、16.0、20.0、25.0 g/kg(组距1:0.8)剂量给小鼠1次性灌胃50%活骨冲剂溶液,给药7日内观察总死亡率。结果全部存活。给药浓度及给药体积均至最大,均未测出1次灌胃后小鼠LD₅₀。

2. 最大耐受量测定:取健康小鼠20只,在24 h内给小鼠灌服50%活骨冲剂溶液2次(每次1.0 ml/只),间隔8 h,总量50 g/kg,相当于成人1次用药量(15 g/50 kg)的166.7倍。经7日观察动物活泼如常,病理检查未见心、肝、肾异常。

讨 论

股骨头无菌性坏死又称股骨头缺血性坏死。其发病原因很多,临床最常见的有严重外伤和大量服用激素两大病因。中医学认为发病多因先天不足,肾气亏损或外伤劳损;六淫侵袭,七情内郁所致的内损等。根据“肾主骨”的理论,活骨冲剂中肉桂、骨碎补、川断、熟地、山萸肉、鹿角胶等补肾之阴阳、强筋骨;黄芪、白芍等益气养血;血竭活血化瘀,消肿止痛;龙骨、牡蛎含钙质较多,能促进血液中Ca、P吸收,达强筋壮骨之功效,我们认为激素为临床常用的免疫抑制剂,长期使用可导致免疫功能失调,引起继发性反应,并影响Ca、P代谢,导致骨质疏松及股骨头无菌性坏死。本结果表明:活骨冲剂能促进血液中Ca、P吸收,同时能促进鸡胚股骨生长等补肾壮骨作用;并有明确的抗炎镇痛作用。本品无毒性作用,是临床上安全可靠,疗效颇佳的药物,利于推广使用。

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into DNA bases, the DNA methylase activity was assayed. The specific activity of DNA methylase was increased by treatment of this drug. The chromatographic behaviour of DNA methylase from rat liver was also changed. These results showed that the mechanism of anti-aging effect of this drug is probably related to DNA methylation.

Key words Bushen Shengxue drug, DNA methylase, Chromatography

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Histological Study on Repairing Experimental Pulp Chamber Floor Perforations with Yunnan Bai-Yao (云南白药) in Dog

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Yunnan Baiyao (YNBY), amalgam, hydroxylapatite and calcium hydroxide were used separately in repairing the experimental pulp chamber floor perforation (PCFP) of dog teeth. The animals were killed at the time of 1, 4, 8 and 12 weeks after experiment. The experimental teeth with periapical tissue were removed and observed their histological changes by optical microscope. The effects that different materials in repairing the tissue of PCFP were observed. Twenty teeth were taken from every dog, each group contained four teeth, the others were used for control group. Results: In initial period (1st, 4th week) inflammatory infiltration was present in PCFP of every group, but the group with YNBY was milder. Pyogenic foci were present in the hydroxylapatite and calcium hydroxide groups. In late stage of experiment (8th and 12th week) inflammatory reactions of each group were alleviated except control group. Epithelial metaplasia and cicatrization were found in the vicinity of perforated area. Dentin cementum and alveolar bone were absorbed in majority of experimental group. It was also observed in the group with YNBY. But in late stage similar cementum formation and alveolar bone neogenesis were found in the perforated area around the repairing materials. It revealed that hemostasis, Promoting Blood Circulation to Remove Stasis and antiphlogistic actions were advantageous to the inflammation, control and repair course of the PCFP. The article discussed that YNBY might promote the repairing of cementum and alveolar bone of perforated area.

Key words periodontal tissue, floor of pulp chamber perforation, histopathology, Yunnan Baiyao

(Original article on page 357)

Experimental Study on Huogu Granule(活骨冲剂) in Treating Aseptic Necrosis of Head of Femur

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The pharmacological activities and acute toxicological activities of Huogu Granule (HGG) on the basis of clinical treatment of aseptic necrosis of head of femur were studied. It was observed that medium and high dose of HGG could reduce the inflammatory reaction, inhibit the enhancement of the capillary permeability and decrease the frequency of body twisting obviously in mice. All of these were significantly better than that of normal saline control group ($P < 0.05$, $P < 0.01$). It showed that HGG had a noticeable anti-inflammatory and analgesic effect. Also HGG was found to improve the absorption of serum Ca, P and the development of femoral growth in chicken embryo. LD₅₀ could not be determined in acute toxicity test, although the dose of 50g/kg · 24hr was about 166.7 times of the dosage used for human adult clinically. It demonstrated that the acute toxicity of HGG is negligible.

Key words aseptic necrosis of head of femur, Huogu Granule, acute toxicity test, pharmacodynamics

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