

实验研究

煨狗骨对实验性骨折愈合作用的 垂体TSH、GH细胞免疫细胞化学研究

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摘要: 为检测煨狗骨促进骨折愈合过程中垂体前叶促甲状腺激素 (TSH) 细胞和生长激素 (GH) 细胞结构和功能变化, 本实验选用家兔54只, 制成双侧桡骨标准骨折模型, 分成煨狗骨用药组和对照组, 术后每周定期取材。脑垂体常规石蜡包埋切片, 行免疫细胞化学染色。结果显示对照组骨折后垂体TSH细胞及GH细胞数量均减少, 而煨狗骨用药组则并未出现对照组的抑制性变化, TSH细胞数量一直维持较高水平, 尤其GH细胞数量随术后时间延长而大幅度上升, 因而说明煨狗骨能促进垂体TSH、GH细胞的功能, 有利于骨折的愈合。

关键词 煨狗骨 骨折愈合 促甲状腺激素细胞 生长激素细胞 实验研究

本文运用免疫细胞化学染色法观察煨狗骨对实验性骨折愈合过程中垂体前叶GH细胞、TSH细胞的变化。

材料和方法

1. 动物选择、骨折造模及分组:

选健康成年家兔54只, 体重1.5~2.0kg, 在无菌条件下制成双侧桡骨中下1/3段3mm缺损的标准骨折模型, 术后随机分成煨狗骨用药组和空白对照组, 分别喂服煨狗骨粉4g/kg/天 (调于20ml生理盐水中) 及等量生理盐水, 术后7、14、21、28、35天以断颈法处死迅速取材。

2. 标本处理及免疫细胞化学染色过程:

(1) 取垂体剥去垂体后叶, 以Bouin's固定24小时后常规石蜡包埋, 冠状切面连续切片(5 μ m)。

(2) 免疫细胞化学染色(ABC法): 切片顺序脱蜡入水, 经3% H_2O_2 处理10分钟后, 磷酸盐缓冲液(PBSpH7.2)冲洗, 入一抗体(抗TSH抗体, 1:800Doako公司; 抗GH抗体, 1:500由Dr. Raiti赠送, NIDDK-NIH) 4 $^{\circ}C$ 孵育过夜, 经PBS冲洗入二抗体(生物素化IgG Vector公司)孵育1小时, 冲洗后入ABC复合物 (Vector公司) 孵

育30分钟, PBS冲洗, DAB显色, 干燥封片。相邻切片作HE对照染色。

(3) 统计垂体TSH、GH阳性细胞数并进行组间差异t检验。

结 果

1. 一般组织学观察结果:

家兔垂体前叶实质细胞排列成条索状, 细胞形态不一, 嗜酸细胞多集中于前叶中间及近垂体中叶部, 嗜碱性细胞较少, 以周边多见, 胞体较小。实质中有较丰富、狭长型的毛细血管, 结缔组织成份较少。骨折后对照组家兔垂体毛细血管增多, 管腔扩大, 而用药组垂体前叶变化较对照组明显, 血窦扩张明显, 并随骨折时间延长, 嗜酸性细胞明显增多, 呈弥漫性分布于整个垂体前叶。

2. 免疫细胞化学染色结果:

促甲状腺激素 (TSH) 阳性细胞和生长激素 (GH) 阳性细胞分布与H.E观察相吻合, TSH细胞约 $13.5 \pm 1.5\mu m$ 大小, 核 $7.4 \pm 0.81\mu m$ 大小, GH细胞为 $11.9 \pm 1.5\mu m$, 核 $7.2 \pm 0.9\mu m$ 。人工骨折手术后对照组TSH细胞数略有增加, 第2周出现明显减少, 但随着骨折术

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后时间延长又逐渐递增,至第5周仍未达到无骨折家兔的激素水平。用药组术后第1周同样大量减少,与对照组相近,但第2周则大幅上升,第3周又大幅下降,与第1周相近,第4周起逐渐增加。对照组骨折后垂体GH细胞在

术后第1周明显升高,术后第2周则大幅下降,从第2周至第5周又出现逐渐递增趋势。用药组则从术后第1周起持续大幅度升高,详见表1、2。

表1 垂体前叶TSH细胞数量变化($\bar{x} \pm S.E$)

骨折后时间	1周	2周	3周	4周	5周
1. 对照组	165.9 ± 118.6	111.0 ± 62.1	117.6 ± 124.9	122.7 ± 48.9	136.8 ± 43.5
2. 用药组	153.4 ± 57.2	384.1 ± 286.5	158.1 ± 72.1	233.7 ± 255.2	272.5 ± 231.3
1、2组间t检验	P > 0.05	P < 0.025	P > 0.05	P > 0.05	P > 0.05

注: ①正常无骨折家兔垂体前叶TSH细胞数为151.6 ± 88.3(个)

②表中数值单位为“个”,下表同。

表2 垂体前叶GH细胞数量变化($\bar{x} \pm S.E$)

骨折后周数	1周	2周	3周	4周	5周
1. 对照组	296.8 ± 126.6	191.1 ± 144.1	213.0 ± 74.6	238.8 ± 69.9	333.4 ± 123.8
2. 用药组	345.6 ± 107.6	417 ± 291.7	683.8 ± 208.8	933.8 ± 356.9	1346.9 ± 339.1
1、2组间t检验	P > 0.05	P < 0.001	P < 0.001	P < 0.0005	P < 0.0005

讨 论

根据本实验结果,对照组TSH细胞数量在骨折后第一周仍维持较高水平,第2周则大幅下降,至术后5周逐渐恢复,这与同期甲状腺结构和功能变化一致^[1]。说明骨折创伤既抑制甲状腺功能,也抑制垂体促甲状腺激素细胞功能,术后第一周轻微升高可能与同期甲状腺功能低下,激素水平较低与反射性刺激有关。用药组术后一直维持较高水平,因而能刺激甲状腺增生,滤泡变小,细胞增高,促进甲状腺功能。术后第2周达到峰值(384.1 ± 286.5)可能与本期骨折断端碎骨片吸收、血肿机化、破骨细胞功能活跃的客观需要有关。甲状腺激素是机体主要的内分泌激素,已经证明能缩短骨的重建周期,刺激皮质骨、松质骨成骨细胞、破骨细胞的活性^[2],增加尿中羟辅氨酸和钙的释放,能升高骨中cAmp含量,增强骨对甲状旁腺激素敏感性^[3]。方氏^[4]实验结果及本实验另一项检测内容即甲状腺结构和功能变化(另文发表)均证明:家兔骨折后甲状腺功能受到抑制,至骨折创伤后第5周才逐渐得以恢复。因此可以认为煨狗骨能逆转骨折对垂体和甲状

腺的抑制作用,并且在较高水平上维持其功能,从而促进骨折局部成骨细胞、破骨细胞活性,促进新骨形成,加速骨折愈合。

骨折后3~5周对照组与用药组组间t检验区别不显著,可能与数值高,而样本含量相对不足有关,事实上两组组间数值相差近一倍。

骨折后对照组垂体的GH细胞数量在较低水平上波动,总体趋势与TSH细胞变化相近。除第一周外,从第2周起逐渐升高,说明对照组骨折术后生长激素的分泌不旺盛,而用药组则呈截然不同表现,随着时间延长,GH细胞数量大幅度上升,证明煨狗骨能明显促进垂体GH细胞功能,促进生长激素的合成和释放,迄今已证明生长激素能直接刺激骨的生长,增加骺板宽度及促进细胞增生和分化^[5]。垂体摘除则导致骺板生长减慢及骨骺发育迟缓。Ray 1972年报道^[6],大鼠桡骨1mm缺损其骨折愈合需12周时间,如果骨折前先行垂体摘除术则延迟愈合,如摘除后施以生长激素治疗则可恢复正常。后来Tylkowski报道^[7]垂体摘除后的骨折愈合过程中骨细胞修复及骨痂形成只是迟缓而未停止,补充生长激素后可产生较多的骨

痂,但主要是软骨性骨痂,只有同时给以生长激素和甲状腺素,才能使骨折愈合过程与对照组(未摘除垂体)同步,说明生长激素与甲状腺素在骨折愈合过程中有协同作用。Koskinen^[9]曾将生长激素和甲状腺素同时用于临床骨折患者,结果发现用药组病人能有更多骨痂形成,也较少发生骨折不愈合。Kumarasiri^[9]运用生化等技术手段证明甲状腺激素能调节生长激素细胞的基因表达及其生长。

本实验结果显示,煅狗骨既促进垂体TSH细胞功能,同时也促进GH细胞的功能,从而增加甲状腺功能,即促进甲状腺激素的分泌。通过甲状腺激素和生长激素的直接间接作用,刺激骨折断端成骨细胞、破骨细胞的活性,促进新生骨小梁形成,最终促进骨折的愈合。

参考文献

1. 王和鸣,等.煅狗骨对实验性骨折愈合作用的甲状腺形态学研究.中国中医骨伤科 1993.
2. Mundy GR et al. Direct stimulation of bone resorption by thyroid hormones. J Clin Invest 1976,58:529.
3. Castro JH, et al. Comparative response to Parathyroid hormone in hyperthyroidism and hypothyroidism Metabolism. 1975, 24: 839.
4. 方一心.家兔实验性骨折愈合过程中甲状腺组织学和组织化学的变化.解剖学报.1966.9(3): 309.
5. Ed'en, S, et al. Specific binding of growth hormones to isolated chondrocytes from rabbit ear, and epiphyseal Plate. Endocrinology 1983,112:1127—29.
6. Rsy RD; The role of Pituitary and thyrod in the nealing of standard bone defeicts Proceed South African Orthop Ass Abstr. In; J Bone Jt Surg 1973,.55B; 442.
7. Tylkowsrki; Cm, et al. Hormonal effects on the morphology of bone defect healing. Clin Orthop 1976,115:274—285.
8. Koskinen EVS, The effect of growth hormone and thyroitropin on human fracture healing. A clinical guantitative, radiographic and metabolic study. Acta Orthop Scand. Suppl 1963,62.
9. Kumarasiri MH. et al. Cell cycle dependence of thyroid hormone nuclear receptors in cultured GC cells. Relationship to Nuclear matrix. Endocrinology 1988,112(5): 1897~2002.

X线微机图像系统对损伤复元糖浆促进骨折后期骨痂生长情况的定量观察

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摘要: 本文作者将70例桡骨远端骨折患者分成损伤复元糖浆试验组与淀粉胶囊对照组,采用X线微机图像系统对患者骨折后第20天、30天、40天所摄X片,进行了骨痂生长、错位程度对骨痂的影响,骨折后继发性骨质疏松情况的定量分析,证实该中药有明显加速骨折愈合的作用,其原理可能与加速机体对骨折端的钙转运有关。

关键词: 中药 骨折愈合 骨痂 X线微机图像系统 实验研究

损伤复元糖浆是我院伤骨科名老中医余靖正祖传接骨验方,已治愈各类骨折患者数万例,疗效显著。为观察该方剂对促进骨折愈合

的确凿疗效,我们采用X线微机图像系统对试验与对照组患者临床治疗中后期的骨痂生长情况进行了定量分析,结果如下。

Abstracts of Original Articles

A cellular immunocytochemical study on pituitary TSH and GH during experimental healing process treated with calcined dog bone

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For determination of the cellular structural and functional changes of the anterior lobe of pituitary thyroid stimulating hormone and growth hormone during the course of promoting fracture healing ability of calcined dog bone, fifty four rabbits were selected to make models of standard fracture of bilateral radius. They were divided into calcined dog bone treating group and control group. Materials were taken for every week postoperatively. The pituitary glands were prepared with paraffin embedding and sections as routinely and stained with immunocytochemical method. The results showed that in control group after fracture, there were decreasing of amount of pituitary TSH and GH cells. There was no such inhibitory changes in the calcined dog bone group. The amount of TSH cellular maintained within a rather high level. Especially the GH cells were elevated in a large scale along with prolongation of the course postoperatively. So it indicates that the calcined dog bone bears the action of promoting the function of pituitary TSH and GH cells and facilitates bone healing.

Key words Calcined dog bone Bone healing

Thyroid stimulating hormone Growth hormone

Experimental study

(original article on page 5)

A quantitative measurement of late stage callus growing treated with Sun Shang Fu Yuan Tang Jiang With X-ray micro-computer figure system

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Seventy cases of distal end fracture of the radius were divided into Sun Shang Fu Yuan Tang Jiang (experimental) group and starch capsule control group. X ray films were taken for 20th, 30th and 40th day after fracture of the patients with X-ray microcomputer figure system. Quantitative measurements were made with callus growing, degree of displacement which influenced callus formation and quantitative analysis of secondary osteoporosis after fracture. It is proved that this Chinese herb has prominent action in accelerating bone healing. The mechanism may be due to acceleration of calcium transportation between fractured ends.

Key words Chinese herbs Fracture healing Callus

X ray micro-computer figure system

Experimental study

(original article on page 7)

Seventy eight cases of cervical spondylosis treated by medicinal pillow

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In this article, medicinal pillow, Tuina and medicine therapy served as observed group, Tuina and medicine therapy as control group. Seventy eight cases of cervical spondylotic patients were observed. The effective rate of medicinal pillow group was 97.56%, and with that of the control group being 83.78%. The therapeutic course of the observed group was shorter than that of the control group. It indicates that the medicinal pillow has the action in helping the recovery of the physiological curve of cervical spine. It can be served as a therapeutic treatment of cervical spondylosis.

Key words Cervical spondylosis Tuina therapy

Chinese medicinal therapy

Medicinal pillow Therapeutic application

(Original article on page 10)

Fracture of femoral neck treated with percutaneous pressor screw

Liu An-qing(刘安庆)et al

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In this article, fifty six cases of fracture of femoral neck were treated with percutaneous pressor screw, the rate of excellent and good was 73%, the rate of non-union, 9%; rate of aseptic necrosis of the femoral head was 17%. The characteristics of the method are small incision, simple to be manipulated. An improving method in taking X ray film of the femoral neck from lateral view was introduced.

Key words Fracture of femoral neck Fracture fixation

(Original article on page 12)

Thirty cases of central type of protrusion of lumbar intervertebral disc treated by mild Tuina under nerve root block anesthesia

Zhou Lin-kuan(周林宽)et al)

Zhejiang College of Traditional Chinese Medicine(310009)

In this article, thirty cases of central and para-central type of protrusion of lumbar intrvertebral disc were treated with mild Tuina under nerve root block anesthesia. The results were cure in 6 cases; marked effectiveness, 9; effectiveness, 6; ineffectiveness, 9; with an effective rate of 70%.

Key words Protrusion of lumbar intervertebral disc Tuina therapy

(Original article on page 24)