

实验研究

# 壮骨素促进骨折愈合的实验研究

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**摘要** 壮骨素及当归、川芎嗪注射液联用,用于家兔桡骨标准骨折模型的治疗。通过体重观察、X片和骨痂切片组织形态计量学分析,结果显示:单纯使用“壮骨素”灌胃治疗组比空白对照组更高的X片评分( $P < 0.001$ )、更高的外骨痂及有效骨痂密度,细胞水平显示活跃的破骨细胞活动( $0.01 < P < 0.05$ )。“壮骨素”与活血化瘀中药联用组早期良好地控制了骨折后动物体重的下降,与对照组相比有非常显著性意义( $P < 0.01$ );同时亦显示更高的X片评分( $P < 0.001$ )、矿化骨痂密度( $0.01 < P < 0.05$ ),破骨细胞活性吸收面( $0.01 < P < 0.05$ )和破骨细胞指数( $P < 0.01$ ),切片显示良好的骨痂改建。

**关键词** 壮骨素 骨折愈合 家兔 实验研究

我们选用中药蚕蛹和蚕砂提取物组成的复方“壮骨素”及当归、川芎嗪注射液联用对骨折愈合的影响进行实验观察、对动物体重变化、X片评分和骨痂计量组织学进行了分析和对照研究。

### 材料与方 法

实验选用封闭群健康大耳白家兔 60 只,体重 3kg 左右,雌雄各半。动物以颗粒型标准饲料喂养,自由饮水。动物按性别、体重均衡后随机分组,3%戊巴比妥钠静脉麻醉,无菌条件下以钢锯造成双侧桡骨中段 3mm 完全缺损,缝合,无菌敷料包扎。

术后动物分为三组,治疗一组以“壮骨素”(浙江省中医药研究院提供)2.2g/kg 体重灌胃,静脉注射生理盐水;治疗二组以“壮骨素”2.2g/kg 体重灌胃,静脉注射当归注射液 0.028ml/kg 体重、川芎嗪 0.14ml/kg 体重;空白对照组以常水灌胃,静脉注射生理盐水。以上用药 1 次/日,全程给药。60 只动物实验中因灌胃致死及双骨折淘汰 18 只,可用标本为 42 个,分 14 及 31 天两相点处死。14 天取材每组 6 只,31 天取材每组 8 只,观察以下指标:

1. 体重变化:记录动物术时体重,及术后

5、7、14、21、28、31 天体重与术时体重差值变化。

2. X 片:取右桡骨骨痂以 70%酒精(4℃)固定,全部材料取完后将骨痂标本于 32 千伏、40 毫秒条件摄 X 光片,同一相点材料摄入同一 X 光片以便对照分析。

3. 骨组织形态计量学分析:取右桡骨骨痂,以 70%酒精固定,甲基丙烯酸甲酯包埋,用 Jung K<sub>3</sub> 硬质切片机制成 5,μm 纵切片,甲苯胺蓝染色,欧波同(OPTON)IBAS 1000 图象分析系统作以下分析测试:

(1)10×10 倍光镜下组织水平计量分析,测定矿化骨小梁骨痂、矿化桥梁或联接骨痂、软骨骨痂、内骨痂、外骨痂、血痂面积,经数学计算三维重建转换成体积密度。

(2)10×40 倍光镜下细胞水平计量分析,测定并转换成以下指标:活性成骨面(类骨质表面成骨细胞覆盖率,OBS%)、成骨细胞指数(每毫米骨小梁表面成骨细胞数目,OBI,个/毫米)、活性吸收面(小梁表面存在破骨细胞的吸收陷窝表面,ARS%)、破骨细胞指数(每毫米骨小梁表面破骨细胞数目,OCI,个/毫米),反映成骨细胞及破骨细胞的数量与活力。

### 结 果

1. 体重观察:从体重相当于术时体重增量

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来看,治疗 I 组动物体重稳定并逐渐上升,但治疗组与对照组骨折后体重均下降,尤以术后 5 天最为明显,三周后恢复术前水平。其中术后 5 天治疗 I 组与对照组相比有非常显著性意义 ( $P < 0.01$ )。

表 1 X 光片累积评分统计分析

组别	积分频数(14天)						积分频数(31天)							
	7	8	9	10	11	12	8	9	10	11	12	13	14	15
治疗 I			2	3	1		1	1	2	1	1	1	1	
治疗 II	1	2	0	2	1		1	1	1	2	1	1		
空白对照	1	1	2	2			2	2	1	2				

2. X 片:采用上海伤骨科研究所 X 片评价标准,通过骨痂投影断端边缘、骨膜反应、骨痂结构、骨痂密度、骨痂边缘 5 项指标评定愈合程度。我们将该标准改进为以 0~3 分计分制

对每项指标评分,将每一标本累积分进行组间非参数分析,结果见表 1。

显示两治疗组愈合均优于对照组 ( $P < 0.001$ )。

3. 骨组织形态计量学分析:

(1)10×10 倍光镜下组织水平计量分析结果见表 2、3。示治疗 I 组 14 天及 31 天两相点外骨痂体积密度均高于对照组 ( $0.01 < P < 0.05$ )。31 天矿化骨痂、矿化桥梁及联接骨痂密度均高于空白对照组 ( $0.01 < P < 0.05$  和  $P < 0.01$ )。治疗 I 组 14 天矿化骨痂密度高于对照组 ( $0.01 < P < 0.05$  和  $P < 0.01$ )。治疗 I 组 14 天矿化骨痂密度高于对照组 ( $0.01 < P < 0.05$ );31 天 t 检验结果与对照组无显著差异。

表 2 骨痂组织水平(10×10 倍光镜)计量分析(14 天) 单位  $\mu\text{m}^3/\mu\text{m}^3$

指 标	治疗 I	治疗 II	空白对照
矿化骨痂	3.74±3.51	3.15±1.68*	1.18±0.56
软骨骨痂	1.12±0.88	2.18±2.09	0.75±0.81
内骨痂	3.46±1.10	6.91±9.70	3.71±1.79
外骨痂	4.29±1.62*	4.79±3.71	1.95±3.54
血 痂	0.046±0.055	0.032±0.04	0.063±0.09

注: \* 0.01 ≤ P < 0.05

表 3 骨痂组织水平(10×10 倍光镜)计量分析(31 天) 单位  $\mu\text{m}^3/\mu\text{m}^3$

指 标	治疗 I	治疗 II	空白对照
矿化骨痂	33.61±28.73*	16.71±10.01	9.27±5.05
矿化桥梁/联接骨痂	14.00±10.74**	5.26±8.25	1.31±1.37
软骨骨痂	2.42±3.70	0.63±0.78	0.46±0.36
内骨痂	2.05±1.26	1.80±1.28	1.24±1.09
外骨痂	17.74±15.97	8.64±12.9	2.30±1.81

注: \* 0.01 ≤ P < 0.05 \*\* P < 0.01

表 4 骨痂细胞水平计量分析(14 天)

指 标	治疗 I	治疗 II	空白对照
活性成骨面(%)	23.646±14.003	35.737±22.836	23.185±15.312
成骨细胞指数(个/mm <sup>3</sup> )	19.446±13.823	27.870±10.320	18.876±14.936
活性吸收面(%)	1.894±1.203	2.794±1.988	5.024±3.24
破骨细胞指数(个/mm <sup>3</sup> )	0.607±0.418	0.963±0.519	1.246±0.527

注:各组间无统计学意义

表 5 骨痂细胞水平计量分析(31 天)

指 标	治疗 I	治疗 II	空白对照
活性成骨面(%)	27.937±11.801	14.362±14.285	20.882±10.771
成骨细胞指数(个/mm <sup>3</sup> )	25.892±9.308	19.508±19.625	19.710±7.256
活性吸收面(%)	3.617±1.834*	3.346±1.817*	1.490±0.301
破骨细胞指数(个/mm <sup>3</sup> )	1.012±0.466*	0.823±0.215**	0.434±0.183

注: \* 0.01 ≤ P < 0.05 \*\* P < 0.01

(2)10×40 倍光镜下细胞水平计量分析结果,表 4、5。t 检验示 14 天组间无显著性差异;31 天治疗 I 组及治疗 II 组活性吸收面及破骨细胞指数均高于对照组(0.01<P<0.05)。

### 讨 论

长骨骨折后由于血管损伤局部迅速形成血肿<sup>[1]</sup>,随着炎性介质释放及单核巨噬细胞活动,引起机体非特异性、急性期反应,表现为低热,负氮平衡,血清铜水平下降<sup>[2]</sup>。失血与蛋白质分解加重了负氮平衡,而血清铜的下降可能是机体抵抗几种铜依赖病原体的感染<sup>[2]</sup>;这些变化均可能对早期骨形成过程产生影响。

与其它组织不同的是,成熟骨是高度有序的组织,骨折导致其序列完全破坏。根据热力学定律,高度有序的建立需要大量的能耗。实验表明,骨折后软骨化骨阶段骨痂中 ATP 含量为正常皮质骨的 1000 倍<sup>[3]</sup>,且多发性骨折病人安静状态下能量消耗比正常人增加 10~20%<sup>[4]</sup>。因此骨折后营养成分的补充比其它组织损伤更为重要。

“壮骨素”复方内含十八种氨基酸、叶绿素等营养成分,及对骨折愈合有促进作用的铜、锌等微量元素。本实验研究的结果表明,“壮骨素”对家兔骨折愈合模型有以下作用:

1. 单纯使用“壮骨素”并未控制骨折后早期体重的下降。但从 X 光片结果来看则优于对照组的愈合。骨痂切片形态计量显示,该组动物 14 天及 31 天切片均显示更高的外骨痂体积密度;31 天切片矿化骨痂、矿化桥梁/联接骨痂体积密度均高于空白对照组。外骨痂起着早期连接和固定断端的作用,外骨痂的存在扩大了骨的外径而增强了骨痂的力学性能,而使骨折能在应力负载下完成<sup>[5]</sup>。另外,该组动物 31 天切片上亦显示活跃的破骨细胞活动。破骨细胞为参与骨吸收的细胞<sup>[6]</sup>,破骨细胞完成骨痂的吸收并引发骨痂的改建,改建使得骨痂在形态及功能两方面达到愈合。单纯使用“壮骨素”

可能对骨折后骨痂形成和改建两方面均起促进作用。

2. “壮骨素”与活血化瘀中药联用良好地控制了家兔骨折后早期体重下降,且体重呈稳定上升趋势,该组结果揭示控制骨折早期体重下降可能不应单纯依靠高营养成分的补充。

X 光片结果与治疗 I 组接近而优于对照组。组织形态计量观察治疗 II 组没有形成明显高于对照组的外骨痂密度,但骨折后 14 天组显示更高的矿化骨痂密度,31 天组显示更高的破骨细胞活性成骨面及破骨细胞指数。从切片形态结合计量学结果,推测该组未形成较大骨痂的原因可能是更为活跃的骨痂改建塑形。该组 31 天切片显示 8 个标本中 2 个已接近髓腔完全再通,这一结果说明该组骨痂改建程度高于治疗 I 组及对照组。本组结果与治疗 I 组的差别是否由于与活血化瘀中药联用,尚有待于进一步的研究。传统中医认为当归、川芎均具有活血化瘀作用,其中当归是古今治疗骨折与创伤的首选药物,但有关作用的药理学基础尚待阐明。

### 参考文献

1. Hulth A. Current concepts of fracture healing Clin Orthop 1989;249:265.
2. Dinarello CA. Interleukin-1 and pathogenesis of the acute phase response. New Engl J Med 1984;311:1413.
3. Leung Ks, et al. Energy metabolism in fracture healing; measurement of adenosine triphosphate in callus to monitor progress. J Bone Joint Surg(Br)1989;71(4):657.
4. Michelson CB, Askanazi J. Current Concepts review. the metabolic response to injury mechanism and clinical implications. J Bone Joint Surg(Am)1986;68-A:782.
5. Perrent SM, Biotzy A. Cellular differentiation and bone biomechanics during the consolidation of fracture. Anal Clin 1978;1:13.
6. Vase G. Cellular biology and biochemical mechanism of bone resorption. An review of recent developments on the formation, activation, and mode of action of osteoclasts. Clin Orthop 1988;231:239.



## Abstract of Original Articles

### Experimental study on Zhuang Gu Su (ZGS) in promoting fracture healing

Xia Zhi-dao(夏志道)

Fang Shi-yuan(房世源) et al

*Institute of Orthopaedics and Traumatology, China Academy of Traditional Chinese Medicine, Beijing (100700)*

Standard fracture model was produced in Rabbits which were divided into three groups to study the effects of ZGS (abstracted from *Bombyx mori* L) and ZGS combined with injections of *Angelica sinensis* and *Lingusticum wallichii*, traditional herbs for treating the fracture. Through observation of body weight changes, the X-ray films, and the histomorphometric analysis of callus section, the results indicated that there were higher X-ray skating ( $P < 0.001$ ), more external callus and effective callus density, more osteoclast activity ( $0.01 < P < 0.05$ ) in ZGS group than the control. In the ZGS combined with promoting blood circulation and removing stasis group, the results showed early controlling of weight reducing in fractured animals ( $P < 0.01$ ) as compared with control, more higher X-ray film skating ( $P < 0.001$ ), mineralization callus density ( $0.01 < P < 0.05$ ), osteoclast activity observing surface ( $0.01 < P < 0.05$ ) and osteoclast index ( $P < 0.001$ ), and the section showed better callus remodeling.

**Key words** Zhuang Gu Su fracture healing rabbit experimental study

(Original article on page 7)

### Experimental study on forging bone in osteogenetic effect

Song Jin-wu(宋进武) et al

*No. 263 Hospital of PLA, Beijing (101149)*

Pig bone was developed into forging bone (true bone ceramic, TBC) through defatting, deproteinization and forging etc. techniques as experimental material in bone transplantation. TBC were transplanted into capsule of rabbit back muscles, hole defect (2.5cm) of tibial periosteal bone and radial shaft. The histological observation taken 2-16 weeks postoperatively indicated that no excessive phenomenon and inflammatory reaction was found. There was active osteogenesis in the reticular formation of TBC. New born bone earlier, quicker and enormous was discovered by fluorescence labelling method. Sixteen within thirty cases were found healed between two fracture ends of TBC and radius after sixteen weeks.

**Key Words** Forging bone osteogenetic effect pig experimental study

(Original article on page 10)

### Dislocation of steuno-clavicular joint

Li Shi-min(李世民)

Wang Lin-sen(王林森) et al

*Tianjin Hospital (300211)*

Thirty—three cases of dislocation of sterno—clavicular joint were reported. Among them, 10 cases were treated operatively; 13, conservatively; 10, without treatment. Within follow—up 20 cases, eight were operated. 5 were excellent and good; 10 belonged to excellent and good within 12 conservative treatment. The authors realized that the successive rate was higher in conservative treatment group in relieving symptoms and recovery of functions. Operative treatment is indicated under special condition.

**Key Words** Dislocation of sterno—clavicular joint close reduction operative reduction  
(Original article on page12)

### **Lumbar intervertebral disc protrusion treated with collagenase lysis**

Jin Xing(金星) Xi Cheng—po(席城坡)et al

*Orthopaedic Hospital of Shen yang, Liaoning(110044)*

Ninty cases of lumbar intervertebral disc protrusion were treated with domestic made collagenase lysis. The effective rate was 93%, the rate of excellent and good being 84%. The authors realized that the method has the advantage of less invasive, non—hemorrhagic and no interference to the spinal canal. So it is an effective therapeutic measure with proper indications and skillful technique.

**Key words** Collagenase lysis lumbar intervertebral disc protrusion  
(Original article on page15)

### **A basic study on communicating branch of L4,5 nerve root**

Liu Jian—shan(刘建丰), du Xin—ru(杜心如)et al

*Affiliated Hospital of Chengde Medical College, Hebei(067000)*

L4,5 nerve root and its communicating branch of 26 adult cadavers were observed. It is realized that the communicating branch of L4,5 nerve root is the anatomic basis of traction test of femoral nerve and scitica and Laseque's sign of the same side. The above signs are specification in protrusion of lumbar intervertebral disc of L4,5.

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