

异种脱蛋白 BMP 复合骨修复骨缺损实验研究

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摘要 异种骨移植常由于强烈的免疫排斥反应失败。本文报告将小牛骨经脱蛋白处理(即脱去主要的抗原物质), 后再复合进去骨形成蛋白(bovine Bone Morphogenetic protein, 简称 b-BMP) 使其成为既无抗原性, 又利于骨形成的异种脱蛋白 BMP 复合骨。将其植入新西兰白兔尺骨缺损(2cm), 观察愈合结果。免疫学、放射学和组织学检查表明, 植入异种脱蛋白 BMP 复合骨, 各组实验动物术后无任何免疫反应。4 周均显示植入骨与骨床界线模糊。8 周植入骨内可见大量成片新生骨细胞及新生血管长入。实验结果表明经处理后的大块异种骨移植, 不但无任何免疫排斥反应, 且可达到预期修复骨缺损的目的。

关键词 骨形成蛋白; 异种骨移植。

采用脱去骨内的部分蛋白, 消除其抗原性并将其(即部分脱蛋白骨)与牛骨形成蛋白(bovine Bone Morphogenetic protein, b-BMP)复合, 既除去异种骨内主要抗原物质—骨内蛋白质, 再复合进去主要成骨物质—骨形成蛋白(BMP)。修复大块骨缺损, 观察修复效果。

材料和方法

1. 复合植骨材料和制备: 取新鲜牛肋骨, 除去附在骨上的软组织及骨膜, 蒸馏水冲洗, 劈成 3×0.5×0.5cm 骨条。按以下顺序处理: 骨条用 3mM NaN₃ 水溶液洗涤, 1:1 甲醇氯仿 4 小时, 0.6N 盐酸 48 小时, 0.5M EDTA 4 小时, 30% 过氧化氢 24 小时。

取 b-BMP (由本所实验室提供)^[1] 15mg 加生理盐水 2ml, 匀浆机搅拌成胶状悬液。将骨条 (3×0.5×0.5cm, 重约 1g) 置入其中, 后将其置抽滤瓶内, 抽吸出植骨块中气体, 使 b-BMP 匀浆液吸入骨块内。低温干燥 (-35℃) 后环氧乙烷空气消毒, 备用 (简称复合骨)。

2. 实验方法: 选用体重 2~2.5kg 之纯种新西兰大白兔 60 只 (本院动物实验室提供), 雌雄不分, 随机分组, 在局麻下 (2% 普鲁卡因 2ml), 无菌手术暴露双侧尺骨中份造成 2cm 的完全缺损, 将复合骨植入缺损处, 1mm×12cm 克氏针固定。术后全部白兔在同样条件下分笼饲养并定期观察 (表 1)。

表 1 异种脱蛋白骨植骨分组情况

组别	左侧 (实验侧)	右侧 (对照侧)
A 组	复合骨	骨缺损区不植骨
B 组	复合骨	自体骨植入
C 组	复合骨	单纯脱蛋白骨

3. 检查方法

(1) 免疫学检查: 术后 2、4、6、12 周从兔耳部采

血, 分离血清, 按 ELISA 间接法, 检测血清中是否有抗牛骨形成蛋白抗体。抗兔的 IgG 抗体为羊抗兔 IgG (批号 92002, 兰州生物所。)

(2) X 线检查: 植骨后 4、8、12、16 周分别对白兔双前肢和定期取材的标本拍摄正位 X 线片, 观察植骨排斥反应, 植入骨的吸收和骨缺损愈合的动态变化。

(3) 组织学检查: 全部白兔于术后 4、8、12、16 周分批处死, 完整取下植骨部位, 用 10% 福尔马林固定, 5% 硝酸—福尔马林脱钙液脱钙 72 小时, 石蜡包埋, 切片经 HE 染色后在光镜下观察。

结 果

术后一般情况良好, 60 只白兔手术伤口均 I 期愈合。

1. 免疫学检查: 按 ELISA 间接法进行检测。在复合骨植入术后 2 周可在部分兔血清中查出抗牛骨形成蛋白抗体。术后 4 周为高峰期。术后 8 周多数兔血清抗体转为阴性。

2. 放射学检查: A 组: 实验侧: 术后 4 周, 移植骨位于骨缺损中, 移植骨与骨床界线模糊; 8 周, 植骨与受骨床界线消失 (图 1); 12~16 周植骨密度近似于宿主骨。对照侧: 各期均呈骨缺损改变。B 组: 实验侧和对照侧各期变化同 A 组实验侧。C 组: 实验侧: 各期变化同 A 组实验侧。对照侧: 植骨 (为单纯脱蛋白骨) 4 周后, 植骨与骨床界线尚清楚; 8 周, 植骨与骨床界线模糊; 12 周植骨与骨床界线仍可见 (图 2); 16 周植骨与骨床界线消失。

3. 组织学检查: 各组实验侧: 植骨后 4 周在植骨与骨床区界未见淋巴细胞和巨噬细胞浸润, 植骨内见大量新生骨细胞呈岛状分布并见有血管长入。8 周植骨内可见大量成片新生骨细胞及大量血管长入 (图 3、



图 2

图 1

为纤维组织。12 周骨折端为成熟骨组织将骨髓腔封闭，缺损处为胶原纤维组织。16 周骨折端为成熟骨组织，缺损处为胶原纤维组织。

对照侧，B 组：4 周骨折处见纤维骨痂组织。8 周骨折处见骨性骨痂组织分布于骨皮质内外侧，但骨皮质外侧较内侧骨痂量多。12 周骨折处为骨性骨痂连接。16 周骨折处骨痂同 12 周，但骨皮质外侧骨痂量较 12 周稍减少。示骨痂已开始吸收塑形。

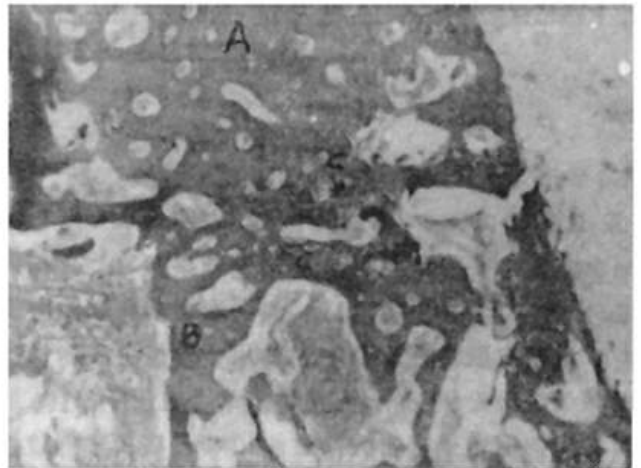


图 5 A 为植入骨；B 为受骨；C 为骨细胞。

对照侧，C 组：4 周骨折线（靠近受骨端）有纤维性骨痂但量较 B 组对照侧少。8 周骨折处见有骨性骨痂组织，但可见少量巢状分布的软骨细胞。12 周骨折端见骨性骨痂组织，但骨痂组织未同植骨连接。16 周骨折处见骨性骨痂组织。骨性骨痂与植骨部分连接。植骨内也未见新生骨细胞。

讨 论

骨移植在治疗骨不连，关节固定以及修复骨缺损中起重要作用。移植骨以新鲜自体骨为优，但有时除不能满足需要外，还造成新的缺损和变形。

异种骨来源广泛。异种骨移植成功的关键环节是异种骨的处理方法。对异种骨的处理，不仅要求减弱植骨的抗原性，还要尽可能保留其成骨诱导能力。国外学者相继制备出冻干小牛骨，脱钙牛骨，Kiel 骨及陶瓷骨等^[2,3]。上述处理虽不同程度的减弱或消除了抗原性，但同时也影响或破坏了骨的诱导力，其异种脱蛋白骨仅能起支架作用^[4,5]。我们利用既没有抗原性又保持同人体骨生理结构相似的脱蛋白牛骨作为支架，将提取的牛 BMP 在真空下与脱蛋白骨复合，使其成为既可成骨，又无免疫反应的脱蛋白 BMP 复合骨。牛骨经脱蛋白处理后，其内已无存活的骨细胞和骨形成蛋白。从实验结果上看，16 周时，C 组对照侧，未见明显的成骨诱导作用。植骨内未见新生骨细胞及新生血管长入。而同 BMP 复合的脱蛋白骨 4 周时，植骨内见小量的新生骨



图 3 A 为植入的异种脱蛋白 BMP 复合骨；B 为新生骨细胞。

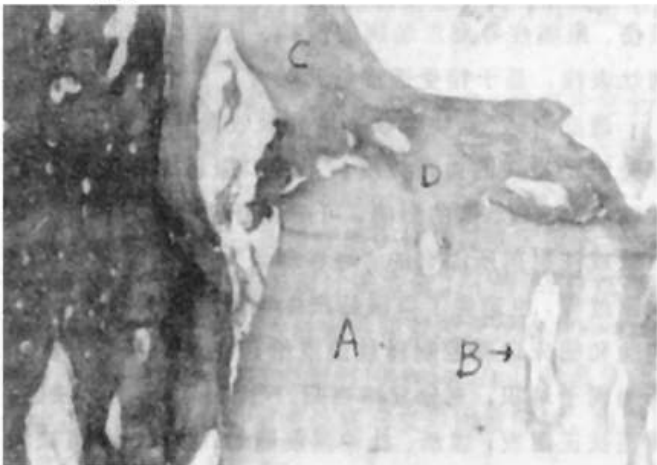


图 4 A 为异种脱蛋白 BMP 复合骨；B 为植骨内新生血管；C 为受骨骨床；D 为受骨床与植骨界线已融合。

4)。12 周植骨与骨床界新生骨钙化成熟，16 周植骨内结构基本同受骨（图 5）。

对照侧，A 组：4 周骨折端见有纤维骨痂组织，缺损处为肉芽组织，8 周骨折端见骨性骨痂组织，缺损处

细胞,16周时植骨内为大片新生骨细胞及血管组织,植骨同受骨床界区已完全达骨性愈合。说明异种脱蛋白骨加BMP,不仅抗原性大为减弱,而且保持了一定的骨诱导性。它植入后,不引起免疫反应,具有填充和支架作用,是一种理想的植骨材料。

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控制释放透皮给药治疗末端病的临床观察

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通过皮肤给药途径的制剂用于骨伤科疾病治疗有着悠久的历史。但是与现代科技结合应用于临床,以达到常规剂型难于达到的疗效,80年代以来才有较快的发展。本文报告控制释放给药系统中的透皮给药治疗末端病的临床应用。

临床资料

治疗组 117 例,其中男 66 例,女 51 例;平均年龄 43 岁;肱骨外上髁炎 56 例,肩袖损伤 18 例,跟痛症 43 例。对照组 56 例,其中男 30 例,女 26 例;平均年龄 47 岁;亦为肱骨外上髁炎、肩袖损伤、跟痛症 3 个病种。

治疗方法

药物组成与制备:当归 黄芪 续断 杜仲 丹参 川芎 玄胡 独活 姜黄 以上药物等量配制成浓度为 30%的酊剂备用。

治疗组:采用广东汕头市 DL-Z 直流感应电疗机,铅质金属电极,电极面积 7.0cm×5.0cm,阴极略大于阳极。将导入上述中药的游子布(生物碱阳离子)放于阳极下,从病灶部皮肤导入,阴极与阳极并置或对置安放好,然后用尼龙扣带固定,接通电源,电流强度以不出现痛感为宜,一般为 5~10mA,每次导入时间为 30 分,每日一次,10 次为一疗程。

对照组:将上述中药煎服,每日一剂,10 剂为一疗程。

治疗结果

评估标准:痊愈:临床症状与体征完全消失;显效:症状与体征基本消失,但劳累活动仍有疼痛感;有效:疼痛明显减轻,功能较有改善;无效:症状与体征无明显改善。

治疗结果:见表。经 1 疗程治疗,治疗组总有效率 94.8%,对照组总有效率 78.6%。两组比较有显著性差异 (P<0.05)。

表 中药透皮给药与煎服一疗程对末端病疗效比较

	痊愈	显效	有效	无效
治疗组	63	36	12	6
对照组	19	14	10	13

讨论

肌腱和韧带在骨上附着点的结构称为末端区。由创伤劳损引起的末端区变性疾患,称为末端病。运动医学和骨伤科领域中的一些疾患,如肱骨外上髁炎、肩袖损伤、跟痛症等属末端病的范畴,且多为牵拉型末端,病灶表浅,易于接受透皮给药治疗。

透皮给药,药物吸收速度和吸收量变化较小,能够保持恒定持久的血药浓度;不像口服药那样受消化道酸碱度,细菌、酶的影响,可避免肝脏的首过作用,无口服或注射剂所固有的“峰谷现象”,大幅度减少药物代谢过程,也避免了口服药产生胃肠道刺激的副作用。实验和临床证明控制释放透皮给药在末端区有大量的药物离子堆积,充分发挥当归、丹参、川芎活血祛瘀,黄芪扶正固表;续断、杜仲强筋健骨;独活、姜黄祛湿散寒止痛的作用,促使末端区疾患痊愈和功能恢复。

透皮给药是施药于外作用于内的一种治疗方法。施药于外作用于内需要一定的动能,中药透皮治疗与电结合,可以借助电的能量促进中药药效由外至内,此外还可以发挥直流电本身消炎消肿效能,二者有同步叠加的双重作用。

(收稿: 1995-03-06)

Abstract of Original Articles

Surgical treatment of the severe thoraco-lumbar burst fracture *Chen Fen-yong, Song Jian-rong, Lin Jia-jun, et al Union Hospital of Fujian Medical College (350001)*

The authors reported 51 cases of severe thoracolumbar burst fracture treated with surgery. According to Frankel grades, there were 13 cases of grade A, 7 cases of grade B, 14 cases of grade C, 5 cases of grade D and 2 cases of grade 3 in 11 cases, and total laminectomy decompression was done in 30 cases. The recovery rate was 73% in the incomplete paraplegia and 15.4% in complete paraplegia. It was concluded 1. Burst fractures mainly injure the middle column of the spinal cord, and spinal canal decompression as well as internal fixation should be done, if the fragments of vertebra had compressed about 1/3 of the spinal canal and the sagittal diameter of the later was less than 10 mm; 2. Internal fixation should be selected according to the condition and range of the injured vertebra, It is reasonable to choose the internal fixation procedure which can cause less injury of the spinal segment and get good results in reduction and fixation; 3. The recovery rate of the incomplete paraplegia group was significantly higher than that of the complete paraplegia group when surgical treatment was applied.

Key words Thoracolumbar vertebrae Burst fracture Surgical treatment

(Original article on page 3)

The following-up analysis on the patients with artificial hip-prosthesis *Zhai Ming-yu, Zhao Yu-gui, Wang Chun-ping, et al. Zhengzhou Hospital of Orthopaedics, Henan Province (450052)*

108 cases (112 hips), applied with artificial prosthesis have been followed up after operation, for the average years of 6.8. It was discovered that 37 cases of complication (about 33%) were produced due to the unproper operation; 46 cases of post-operational complication (41.1%); and the satisfactory therapeutic effective rate being about 74.1%.

The frequently encountered reasons and treatments of the various kinds of complications were put into stress

to be analysed and discussed in this paper.

KEY WORDS Artificial prosthesis Disease of the hip region

(Original article on page 5)

Study of the effect of intermittent compressive pressure to the osteoblasts in vitro. *Li Ke-xin, Shang Tian-yu, Dong Fu-hui, et al. Institute of Orthopaedics & Traumatology, Chinese Academy of TCM (100700)*

The experiment imitated the physiological changes of the cellular external circumstances, existed during skeletal functional movement, and supplied a intermittent compressive pressure (0.098 MPa, 15 minutes pressure, 15 minutes relax, 2 cycles/one hour, 8 hours/day) to the osteoblasts of experimental groups in vitro. It was discovered that the numbers of osteoblasts and the reaction of alkaline phosphatase in the experimental group were markedly elevated than that of the control groups. The results indicate that the intermittent compressive pressure is able to improve the proliferation and differentiation of the osteoblasts.

KEY WORDS Intermittent compressive pressure Osteoblast in vitro

(Original article on page 7)

Experimental research on the restoration of bone defect with the complex of heterogenous deproteinized bone and the bone morphogenetic protein. *Bai Meng-hai, Ge Bao-feng, Wang Yong, et al. Institute of Orthopaedics & Traumatology, Lanzhou General Hospital of the Military Region (730050)*

The failure of the implantation of the heterogenous deproteinized bone is always due to the intensive immune rejection. A new method for treating heterogenous bone was described in this paper. The bone of calf was deproteinized, i. e. extracted the main antigens and combined with bovine bone morphogenetic protein (BMMP) and then produced a kind of heterogenous deproteinized bone, not only without antigenicity, but also advantageous to the bone formation. Implanting this kinds of bone complex into the artificial defect (2cm) of the radius of Newzeland rabbit, the observation on the recovery with immunological, radioactive, and histological

methods, demonstrated that there was without any immune rejection in various kinds of experimental groups and there was indistinct margin between the implant and the fracture bed at 4th week; There were a great amount of lamella of new osteocytes and neogenetic vessels invasion into the implant, at the 8th week.

The experimental result indicates that the large heterogenous bone implantation, treated beforehand, can not only without any immune rejection, but also can achieve the expected restoration.

KEY WORDS Bone morphogenetic protein
Heterogenous bone implantation

(Original article on page 10)

Evaluation of the effect of ALQ on the experimental spinal cord injury, by means of evoked potential in the later.

pt. of Physiology, Anhui Medical University (230032)

Three kinds of experimental model of spinal cord injury (light, severe and complete) had been imitated in sixty rabbits and part of them were treated by autohemotherapy of light quanta (ALQ). The effect of ALQ on paralysis was observed and reported, by means of spinal cord evoked potential (SCEP), a reliable electrophysiological index. The results demonstrated that the percentage of the recovery of SCEP in the incomplete spinal injury of the treated group, was higher than that in the control group. It indicates that ALQ can promote the functional recovery of the spinal cord from the damage.

KEY WORDS Spinal injuries Evoked potential
Autohemotherapy

(Original article on page 13)

Techniques of needle manipulation for the treatment of metacarpophalangeal joint dislocation *Ren Qi-uang, Gao Min. Department of Orthopedics, The First Affiliated Hospital, Anhui Medical University (Hefei 230022)*

The closed dislocation of the metacarpophalangeal joint is not common. The open-reduction is often needed to be done, owing to the speciality of its anatomical relationship. Here an experience of close reduction in

success with prying and plucking manipulation, applied with Kirschner needle on 30 cases was introduced in this paper. The mechanism and procedure of this method was demonstrated with anatomical data, diagrams and typical cases and its practical value was also discussed.

KEY WORDS Prying and plucking manipulation with Kirschner needle Dislocation of metacarpophalangeal joint.

(Original article on page 15)

The Treatment of teno-skin suture on 54 cases of mallet finger deformity *Li Liang-dong, Fang Ming-zhi, Shen Jun, et al. First Affiliated Hospital, Guiyang College of Traditional Chinese Medicine (550001)*

54 cases of fresh and old mallet finger deformity have been treated with the combination of the teno-skin suture and small splinter fixation and achieved satisfactory therapeutic effect. Analysis, according to the characteristics of the anatomy and mechanics of this kind of operation, indicated that this pattern of operation is the first choice for the middle and old aged patients.

KEY WORDS Mallet finger Small splinter fixation

(Original article on page 17)

The Demonstration of the functional digits of brachial plexus roots avulsion. *Pei Lian-kui, Liang Bing-sheng, Zhang Jian-zhong.*

Department of Orthopaedics, Second Affiliated Hospital of Shanxi Medical College, Taiyuan (030001)

In 1993, we had reported the functional digits demonstrated at different parts of upper-limbs. Now we demonstrate the digits and percentages of function on the patients attacked with brachial plexus roots avulsion. The results indicate that the digital demonstration in the orthopaedics is an useful quantitative method to identify the injuries and curative effect on the patients and it is more standardized, objective and distinctive to evaluate the clinical curative effects.

KEY WORDS Functional digits Brachial roots avulsion Orthopaedics

(Original article on page 36)