

实验研究

骨折愈合中的软骨骨痂

——形态学演变及超微结构观察

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摘要 通过光镜下不脱钙骨组织学、组织化学和透射电镜对雄性兔桡骨标准缺损骨折模型愈合中软骨骨痂的形成、演变及超微结构进行了观察。结果显示：软骨骨痂由骨折后进入断端间的肉芽组织分化而成，其形成和改建并不完全相同于骺板软骨内化骨。电镜下，骨痂内软骨细胞可分为 5 个发育阶段：成软骨细胞、软骨细胞、肥大软骨细胞、变性软骨细胞和残存软骨细胞。我们认为 1) 软骨骨痂由断端周围组织内的间充质细胞分化而成；2) 在改建过程中，软骨骨痂能直接形成骨小梁，我们支持肥大软骨细胞能转化为骨细胞的假说；3) 软骨骨痂对骨折愈合有重要的作用，能早期填充骨缺损，联接断端，是骨折在重力负载下完成愈合的基础组织之一。

关键词 软骨骨痂 骨折愈合 形态学

为进一步研究软骨骨痂在骨折愈合中的作用，我们对家兔桡骨标准缺损骨折模型中软骨骨痂的形态学演变及软骨细胞的超微结构进行了观察，报道如下。

材料与方法

封闭群健康日本大耳白雄性家兔 16 只，体重 2.5~3kg，在 3% 戊巴比妥钠静脉麻醉下造成两侧桡骨中段 3mm 完全缺损，缝合创口，颗粒型标准饲料喂养。动物材料分别用光镜及透射电镜观察。

光镜标本制备：于骨折后 1、3、5、7、14、31、49 天分别取右侧桡骨骨痂，70% 酒精 4℃ 固定，甲基丙烯酸甲酯包埋，Jung K₃ 硬质切片机切片，甲苯胺蓝及 Masson—Goldner 三色法染色，光镜观察。左桡骨骨痂以丙酮 4℃ 固定，甲基丙烯酸甲酯低温(4℃)包埋，以偶氮偶联法示碱性磷酸酶及酸性磷酸酶。

电镜标本制备：取骨折后 14 及 31 天两相点左桡骨骨痂，以 3% 戊二醛固定，EDTA 脱钙，1% 锇酸后固定，Epon812 包埋，超薄切片，醋酸铀、枸橼酸铅双重染色，JEM1000EX 透射电镜观察。

结果

1. 光镜观察

不脱钙骨痂切片上，甲苯胺蓝染色很容易区分出骨痂中不同基质而将矿化骨、类骨质、纤维基质及成熟程度不同的软骨基质区分开来。

骨折后 24 小时可见断端内外骨膜成骨细胞活动，但断端间仅为血肿填充。

骨折后 3 天，断端周围组织包括断端内外骨膜均有大量细胞增生，部分成纤维细胞样梭形细胞向断端间移行并分泌纤维基质。

骨折后 5、7 天，增殖细胞与新生小血管形成肉芽组织，早期移行的细胞其前锋已进入机化血肿中。骨折 5 天细胞密集区出现软骨基质样着色，骨折 7 天外骨痂中出现小片幼稚软骨细胞。

骨折后 14 天，骨缺损区中轴已为软骨骨痂联接，但尚未填满缺损区，偶见纤维组织及血肿残留。软骨骨痂与纤维组织之间可见大量梭形细胞加入软骨并分泌软骨样基质，而后由梭形变成接近圆形，细胞核逐渐饱满，胞质嗜碱性增强，基质着色加深(图 1，见封 2)。与此同时软骨骨痂与骨性骨痂之间可见软骨的钙化与改建。钙化软骨基质着色加深接近矿化

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骨,部分细胞肥大及死亡崩解(图 2,见封 2)。

骨折后 31 天,骨痂切片中已无新形成的软骨,愈合的骨膜将梭形外骨痂与纤维组织分隔开,骨痂软骨内化骨达到高峰。不同部位的软骨均出现钙化,随着破骨细胞活动及新生血管长入,成骨细胞直接在矿化软骨表面成骨,在骨基质与软骨基质之间缺乏粘合线,因而新沉积的骨细胞与残存的软骨细胞邻接(图 3,见封 2)。

骨折后 49 天,已完全愈合的标本已不再能见到软骨基质痕迹,仅少数标本可偶见数个陷窝壁呈软骨基质样着色的细胞存在于改建后交织骨中。

组织化学显示,肥大软骨细胞呈碱性磷酸酶强阳性,但幼稚软骨缺乏该酶活性。

2. 电镜观察

依据超微结构的差异,可将软骨细胞区分为五种形态,分述于下。

(1)成软骨细胞:为扁平梭形细胞,位于纤维基质与软骨基质之间。胞核扁圆形,胞质较少,内含线粒体、粗面内质网及较多的糖原颗粒。细胞表面有短小的突起,周围有其分泌的细的胶原纤维及蛋白多糖样颗粒(图 4,见封 2)。

(2)成熟软骨细胞:细胞轮廓从梭形至接近圆形。胞核扁圆或圆形,可见 1~3 个核仁。胞质富含发育良好的高尔基复合体、粗面内质网、线粒体及分泌小泡。细胞表面有不规则的凹陷及短小的突起(图 5,见封 2)。

(3)肥大软骨细胞:细胞外形饱满,胞核肿胀而呈圆形,核质固缩,胞质内细胞器减少而含空泡结构,细胞表面出现较长突起,陷窝壁基质呈致密均质结构。

(4)变性死亡软骨细胞:胞核肿胀或皱缩,细胞器崩解,细胞膜结构消失而与基质混淆。最终细胞轮廓亦消失而留下空洞的陷窝。

(5)残存软骨细胞:其外形介于肥大软骨细胞与骨细胞之间。胞核呈饱满圆形,胞质中细胞器较少,含有空泡结构。细胞表面出现类似骨细胞的较长突起(图 6,见封 2)。

讨论

1. 软骨骨痂的形成

关于软骨骨痂形成的影响因素,以往广为接受的想法是(1)低氧分压(PO_2);(2)血供不良;(3)断端固定不稳。软骨骨痂的产生被认为是治疗方法的不当。

最近这一经典看法受到挑战。首先,软骨中氧分压并不比小梁骨更低^[1];其次,骨折后断端周围毛细血管均有十分活跃的增生^[2],且 Hult 通过免疫组化标记显示软骨骨痂中残留血管样结构而指出软骨骨痂形成于富含血管的肉芽组织而非血供不良^[3];最后,在骨形态发生蛋白异位诱导成骨时没有固定因素的影响,仍然形成骨细胞及软骨内化骨,说明其产生并非断端不稳。

从我们的观察看,来自外骨膜及周围肉芽组织的梭形细胞进入缺损区后均分化成为软骨而无例外。推测软骨的前体细胞向软骨分化更可能的原因为局部微环境因子,而使可诱导的骨祖细胞向软骨方向分化^[4,5]。这些因子除了骨诱导蛋白外,断端间炎症反应释放的细胞活素亦起十分重要的作用^[6]。

2. 软骨骨痂的转归

骨折完全愈合后不再存有软骨骨痂,软骨仅为长骨骨折愈合的中间阶段,经软骨内化骨而被吸收替代。

骨痂软骨内化骨与骺板类似^[6]但有明显的差别:(1)软骨骨痂没有软骨膜及生发层,成软骨细胞来自纤维肉芽组织;(2)软骨骨痂在多个部位同时钙化,且其吸收替代在多个部位和方向进行;(3)软骨骨痂改建时新骨直接沉积于钙化软骨表面,两种基质的界面缺乏粘合线,因而骨细胞与肥大软骨细胞并存于同一小梁而没有界限。

尽管软骨钙化后肥大软骨细胞大多变性死亡,但仍有相当一部分因与改建中新沉积的骨细胞相邻而幸存下来,且形态及超微结构均有向骨细胞衍化的趋势。因此,我们支持肥大软骨细胞能转化为骨细胞的假说^[7]。

3. 软骨骨痂在骨折愈合中的地位

骨折愈合一度被分为两种方式,即 I 期愈合和 II 期愈合。I 期愈合是指坚强内固定时缺乏软骨骨痂的一种人为条件下的愈合方式,由于应力遮挡效应及外骨痂的缺乏易致再骨折⁽⁹⁾。事实上,包括坚强内固定在内的各种长骨骨折,其愈合或多或少均有软骨骨痂参与。以软骨骨痂为中间过程的骨折愈合至少有以下优势:(1)软骨骨痂可来自骨诱导,因而有更为广泛的细胞来源;(2)软骨骨痂能在短期内充填骨缺损从而维持断端相对稳定;(3)梭形外骨痂稳定断端的同时增加了骨痂截面积,从而增加了其力学性能⁽⁹⁾,且软骨的粘弹性增加了骨对破坏性应力的耐受而使得骨折愈合能在应力负载下完成。

尽管软骨骨痂具有以上作用,但软骨骨痂并不能代替骨组织,其不足是显而易见的。可以预见,随着治疗方法的改进,软骨骨痂出现的量与度及其改建将可调控,从而使骨折在时间与质量两方面均达最佳愈合。

附:图片说明图①纤维组织中梭形细胞分化为软骨细胞。图②软骨骨痂的钙化,右侧为肥大软骨细胞,左侧为钙化软骨。图③软骨骨痂的改建,成骨细胞直接在钙化软骨表面成骨,中央部分小梁中为残存的软骨细胞。图①~③均为甲苯胺蓝染色,×400。图④纤维组织中分化的成软骨细胞,示细胞表面短小的突起及基质中蛋白多糖样颗粒,×6,000。图⑤成熟软骨细胞,高度发育的高尔基复合体,粗面内质网及典型的

软骨基质结构,×6,000。图⑥残存软骨细胞,周围空洞为死亡软骨陷窝,×5,000。

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《点压特定穴位治疗小儿脑瘫》出版

由陆文琴等编著的《点压特定穴位治疗小儿脑瘫》一书,已由中医古籍出版社出版。书中以图解的形式,详细介绍了小儿脑瘫的发病原因、小儿特定穴位、常用点压操作方法和小儿脑瘫的康复治疗。全书每册 6 元(含挂号邮资),需要者可与中国中医研究院骨伤科研究所陆文琴联系(地址:北京市东直门北新仓 18 号,邮政编码:100700)。

Abstract of Original Articles

Clinical and experimental studies on spondylolithiasis treated by manipulation

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Displacement microsensors were applied to measure fresh cadaver specimens in mimic the displacement in movement and rotatory reduction. It was found that the inferior facet joint of the lumbar spine producing an upward and lateral displacement to enlarge the intervertebral foramen, the vertebral body producing a tendency of relatively backward and retaining to its original position. Through naked eye observation, there was increasing of the width of the isthmus under the action of manipulation of the gap of isthmus. Thirty-five cases of spondylolithiasis were treated by manipulation with satisfactory results, especially for those suffering with degenerative type. It showed that lying in supine position with flexion of knees and hip joints, by rolling the waist, sitting up exercise and iso-tension training are important procedures in therapy.

Key Words Spondylolithiasis Bio-mechanics Manipulation Iso-tension training

Clinical analysis of twenty-two cases of tower-door type of fracture of ankle joint

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Tower-door type fracture of the ankle joint is a rare and intra-articular fracture which is difficult to be treated. Twenty-two cases of such kind of fracture were treated with simple manipulative reduction and application of plaster of Paris as external fixation, prizing dispelling reduction and internal fixation with steel wire, steel wire internal fixation via open reduction, fusion of the ankle joint etc. Better results were gained with prizing-dispelling reduction and internal fixation with steel wire via open reduction. But simple manipulation with plaster of Paris as external fixation and joint fusion had worse results. Pathogenesis of the fracture diagnosis, therapeutic etc. problems were discussed.

Key Words Intra-articular fracture Reduction of tower-door type fracture Internal fixation with steel wire Joint fusion

Cartilage callus in bone healing

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Through light microscopic non-decalcified bone histological, histochemical and electron microscopic observations on formation, evolution and ultrastructure of cartilage callus of rabbit radial bone fracture standard defect model during healing process, the results showed that the cartilage callus comes from differentiation of granular tissue of the fracture ends, its formation and reconstruction did not completely similar with ossification in the epiphyseal chondrium. There are five evolutionary stages of cartilage callus cells, the callus under electron microscopic

findings, i. e chondroblasts, chondrocytes hypertrophic chonocytes, degenerative chonocytes and remnant chondrocytes. We realized that 1)the chondrial callus is differentiated from interstitial cells surrounding the fracture end ,2)during reconstruction process, chondrial callus can directly form bone trabeculae. We support the hypothesis that hypertrophied chonocytes. can transfer to bone cells,3)chondrial callus bears important action during healing process. it can fulfil bone defect in the early stage, connect fracture ends, the fracture healing process is completed under the burden of gravity.

Key Words Cartilage callus Fracture healing Morphology

Experimental and clinical observation on Gao Wu Jia Su Shang Cha Ji in treating acute soft tissue injury

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Externally applied Gao Wu Jia Su, extract of Chinese herb Gao Wu Tou frost ointment was introduced to treat acute soft tissue injury . Through an observation of animal experiment and 500 patients ,the results indicated that the drug bears better dispelling action of edema, analgesic action and dispelling stasis, and it has no toxic and side—effect. The total effective rate reaches 99. 2%. It is easy to be applied ,so it is an ideal external used drug in treating acute soft tissue injury.

Key Words Soft tissue injury Gao Wu Jia Su External therapeutic method with Chinese herb Experimental study Clinical appilcation

Clinical observation of elastic external fixation and functional exercise under burder in treating non—union of tibia

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

Li Kexin et al (李可心)

Twenty—two cases of non—union of tibia treated by external fixation with wire and functional exercise under burder. Among them, 10 cases were proliferative type, 12 atrophic type. The causes of tailure are 11 cases for defext in internal fixation, 4 for plaster of Paris fixator, 2 for self—made splint fixation with bad fixation , and 5 for infection of the fracture ends. Through a course of therapy from 87—189 days, with an average of 103 days, 19 cases healed within months ,3 with worse effectiveness. The result of analysis showed that, the maincause of non—union was due to insufficient therapy interference to natural healing process, with limitation of the ability of bone growth. Elasticexternal fixation can improve the stability of fracture ends, offer elastic and interrupted physiological pressure stress. It dons't limit the blood supply action of the muscle pump during functional exercies. It facilitaes recovery the ability of growth of the fracture ends .

Key words Non—union External fixator for fracture