实验研究

骨痂中多核巨细胞与破骨细胞的 组织学及超微结构观察

中国中医研究院骨伤科研究所(北京 100700)

常超英 夏志道* 房世源 孙树椿 王来鸿 吴非** 时光达**

摘要 本文通过光镜与透射电镜对家兔桡骨标准骨折模型骨痂内多核巨细胞与破骨细胞形态进行了观察。 观察显示,两种细胞均参与骨吸收,但多核细胞主要在骨折早期吸收死骨和骨碎屑,吸收方式包括吞噬和细胞外降解;破骨细胞主要吸收钙化软骨骨痂和新生骨小梁,通过细胞外吸收方式完成骨痂改建。作者认为,多核巨细胞表面的丝状伪足和破骨细胞表面的皱折缘与骨矿物质细胞外降解有关,但其降解机理的差别尚有待揭示。

关键词 骨痂 组织学 超微结构

作者通过骨痂切片, 对家兔桡骨标准骨折 愈合模型中的多核巨细胞和破骨细胞进行了 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, 体重 3kg 左右, 3% 戊巴比妥钠静脉麻醉下手术造成双侧桡骨中段 3mm 缺损, 随即缝合创口。术后取 14 天及 31 天骨痂, 分别用作光镜和电镜标本制备。

- 1. 光镜: 取右侧桡骨骨痂, 70%酒精 4°c 固定, 逐级酒精脱水, 二甲苯透明, 甲基丙烯酸甲酯包埋, Jung K3 硬质切片机切制 5 μm 切片, 甲苯胺蓝及 Masson Goldner 三色法染色, 光镜观察。
- 2. 透射电镜: 取左侧桡骨骨痂, 3%戊二醛固定, 10% EDTA 脱钙, 1%OsO4后固定, Epon 812 包埋, LKB5 型超薄切片机切片, 醋酸铀、枸橼酸铅双重染色, JEM1200EX 透射电镜观察。

结果

1. 多核巨细胞: 多核巨细胞光镜下可见 2 ~15 个核,核型不规则,胞质着色较浅。一个或 数个多核细胞将骨碎屑、死骨片包绕或吞噬 (图 1 见封 3)。电镜下多核巨细胞可区分为两种形态,即多核巨噬细胞 (multinuclear macrophage) 和异物巨细胞 (foreign body giant cell)。

多核巨噬细胞直径约 10~30 µm, 外形不规则, 有 2 个以上的细胞核, 核多呈圆形或扁圆形, 可见核膜凹陷, 偶见核仁 (图 2 见封 3)。细胞器比单个核巨噬细胞丰富且发育良好。胞质中富含线粒体、粗面内质网、高尔基复合体、溶酶体及较多的微丝样结构。多核巨噬细胞的胞核间无膜样结构间隔, 可见活跃的吞噬活动, 胞质中可见大小达 4.1 µm×6.5 µm 的吞噬体, 以单层界膜与胞质分离, 细胞表面有较多突起。

异物巨细胞直径达 30 μm 以上, 可见 2~15 个细胞核, 核形不规则, 核膜常有较多凹陷, 核质均匀, 可见核仁。胞质中可见线粒体、粗面内质网、糖原颗粒、微管、微丝及大小不等的空泡(图 3 见封 3)。胞核之间可见复杂的膜折叠样结构。细胞表面可见微绒毛样丝状伪足(filopod), 其横径约 20 μm, 长约 2~5 μm。骨碎片被异物巨细胞长达 10~30 μm 的突起包绕, 丝状伪足则位于这些突起的表面。电镜下可见骨碎片的分隔、碎裂和崩解, 但未显示这

同济医科大学附属协和医院

^{• •} 贵阳中医学院骨伤科研究所

些颗粒的吞噬(图 4 见封 3)。

2. 破骨细胞: 破骨细胞常见为多核的巨型 细胞, 存在于骨表面的吸收陷窝 (Howship's Lacunae)中。光镜下可见 2~15 个胞核、胞质 弱嗜碱性,其与骨组织接触区着色更浅并呈泡 沫样、骨面可见由于钙脱失而失去矿物质着色 (图 5 见封 3)。电镜下可见单个核的前破骨细 胞及多核的成熟破骨细胞。成熟破骨细胞直径 可达 30 4 m 以上, 细胞核呈圆形或扁原形, 核 膜有较多凹陷、核质均匀、可见核仁。胞质富含 线粒体及游离核糖体, 亦可见粗面及滑面内质 网、高尔基复合体及较多的空泡、空泡多位于 靠近骨吸收面的胞质中。典型的破骨细胞可见 清亮区 (clear zone) 和皱折缘 (ruffled border)。清亮区位于破骨细胞与骨接触区的 周缘、与骨组织紧密接触、表面较少突起、胞质 中缺乏细胞器而呈透明状, 该区可见起于细胞 膜而向胞质延伸的微丝样结构 (图 (见封 3)。 皱折缘为骨吸收面的细胞膜折迭而成, 形成手 指样突起伸入骨基质中, 突起长约1~2 µ m, 横径约100~200 µm、表面有纤细的毛茸茸样 结构、突起内有较多小泡、直径约10~20 µm。

讨论

本文作者观察到, 骨痂中多核巨细胞与破骨细胞均参与骨矿物质成分的吸收, 但多核巨细胞主要出现在骨折早期断端间残留的骨碎屑及死骨区, 而破骨细胞则出现在新生骨小梁及矿化软骨基质表面, 参与骨组织及矿化软骨基质的吸收。

多核巨细胞对死骨和骨碎屑的吸收采用两种方式,即吞噬消化和细胞外降解,这与Rifkin体外培养观察的结果一致¹¹。对于较小的颗粒,由多核巨噬细胞进行吞噬。对于较大的碎片则由多个巨噬细胞融合成异物巨细胞而进行细胞外降解吸收。多核巨噬细胞与异物巨细胞的区别在于: 前者细胞核之间没有细胞膜样间隔,推测其形成于核分裂,而且具有较强的吞噬能力; 后者细胞核之间有细胞膜样间隔, 形成于细胞融合, 不具备吞噬能力。异物巨

细胞骨吸收表面的丝状伪足可能与细胞分泌和吸收有关,而在骨无机成分细胞外降解中发挥重要作用。由于巨噬细胞能分泌胶原酶^[2],因此能完成骨基质成分的降解。此外,骨基质成分能诱导单核一巨噬细胞系细分泌白细胞介素 1 等炎性介质和细胞活素,从而调节骨吸收与骨形成。^[3,4]

破骨细胞对骨组织的吸收过程为激活的破骨细胞以清亮区密接于骨面而形成一个封闭的吸收区,推测其脱钙过程为破骨细胞皱折缘上的质子泵将细胞内碳酸酐酶解离碳酸形成的 H[†] 泌出细胞外, 造成局部低 ph 值而使骨脱钙, 并完成骨盐的吸收。一般认为破骨细胞不具备吞噬能力, 也不完成骨胶原的吸收。对钙化软骨吸收过程与此类似。吸收完成后, 吸收区为成骨细胞的成骨活动所替代。

骨折早期由于断端血管损伤,哈佛氏系统封闭,断端间为血肿填充,活骨组织内的破骨细胞不可能到达死骨区域,此时死骨及坏死组织的吸收则可能由具有游走能力的巨噬细胞及其演变的多核巨细胞完成。随着坏死组织的清除,软骨钙化及新生骨小梁的形成,来自新生血管的破骨细胞开始进行矿化骨痂的吸收改建,多核巨细胞则随着死骨吸收的完成而从断端间消失。至于多核巨细胞是否具有类似于破骨细胞的泌 H⁺功能,及其在骨盐吸收机制上的差别,尚待进一步实验揭示。

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Abstract of Original Articles

Clinical study on epiphyseal injury treated by integration of traditional Chinese and modern medicine

Zhu Shiyi (朱式仪) Gu Yunwu (顾云五)

Tianjin Hospital, Tianjin City (300211)

A new classification of epiphyseal injury was suggested through clinical and X-ray analysis of nine positions in epiphyseal injury on extremities in 363 cases, in the meanwhile pathogenesis of epiphyseal injury, morphology, classification, reatment and prognosis etc. problems were clarified. A preliminary exploration of therapeutic theoretical basis and prin ciples were made via a remoto observation of 216 cases, the rate of therapeutic excellant and good with maneuver reduction and splint fixation was 91.1%. It was prominently superior than open reduction of which the efficacy was 71.9%. Integration of traditional Chinese and modern medicinal therapy fits to various age, position and kinds of most epiphyseal injuries.

Key words Epiphysis Trauma and injury

Integration of traditionl Chinese and modern medicinal therapy

(Original article on page 5)

Multinclear giant cells and osteoclasts in callus - histological and ultrastructural observation

Chang Chaoying (常超英) Xia Zhidao (夏志道) Fang Shiyuan (房世源) et al Institute of Orthopaedics and Traumatology, China Academy of TCM, Beijing 100700; Institute of Orthopaedics and Traumatology Guiyang College of TCM, Guiyang 350001

The morphology of multinuclear giant cells (MGC) and osteoclasts (Oc) in rabbit's radius callus had been observed by light microscopy and transmission electron microscopy. It showed that both MGC and Oc participated in bone resorption, but MGC mainly resorbed dead bone and bone chips through phagocytosis and extracellular degradation at the early stage of fracturehealing, and Oc mostly resorbed calcified cartilaginous callus and new formed trabecular bone by extracellular resorption to accomplish the remodeling of callus. It is suggested that the filopods on the surface of MGC and ruffled border on the surface of Oc are closely related to extracellular degradation of bone mineral. Their difference in the mechanism of degradation is waiting or further study.

Key words Callus Histology Ultrastructure

(Original article on page 8)

Influence of ultrastructure of rabbit skeletal muscle mimic mountain - climbing myalgia treated with Yue Ji Ling lotion

Li pengtao(李澎涛) Wang Xinyue (王新月) Zhang Wensheng (张文生) et al Hubei College of Traditional Chinese Medicine (050091)

Exploration of the pathology of mountain—climbing myalgia and observation of therapeutic action of Yue Ji Ling Lotion on rabbit with over burden motion mimic human mountain—climbing action were carried out. The results indicated that the functional state of rabbit extremities is similar to human mountain—climbing myalgia and the ultrastructure of skeletal muscle showed prominent traumatic changes. Yue Ji Ling lotion bears the function of inhibition the traumatic changes effectively and restoration the normal function of the limbs as well.

Key words Skeletal muscular system Yue Ji Ling lotion Ultrastructure
(Original article on page 10)

Dr. Shi Weizhi's experience in treating cervical spondylotic myelopathy Mao Xiao (茅晓)

Shanghai Institution of Traditional Chinese Medicine (200032)

Old traditional Chinese medical doctor Shi Weizhi holds that the pathogenesis of cervical spondylotic myelopathy is due to insufficiency of liver and kidney, emptiness of Du meridian, blockade of the meridian due to wind-dampness, obstruction of flowing of Qi and blood. Better results were obtained after application of the therapeutic principles of invigorating the liver and kidney, warming the meridian, invigorating of Qi and activating of blood circulation, and expelling of wind and dredging the meridian passage.

Key word Cervical spondylosis Pathogenesis

Traditional Chinese medicinal therapy

(Original article on page 12)

Study and clinical application on frame-style ladder form enhanced steel plate

Chen Dongan (陈东安) Ge Baofeng (葛宝丰) liu Xingyan (刘兴炎) et al Lanzhou Amry General Hospital, Institite of Orthopaedics (Gansu)

Based on the principle of bio-mechanics and starting point on he stability if internal fixation, double arm frame-style ladder form enhanced steel plate was designed. The therapeutic effect was satisfactory after 37 cases had been used clinically. No one case of break wire, pulling out wire and refracture enomenon on the fractured end was found during withdrawing the steel plate.

Key words Fracture fixation Frame-style ladder form enhanced steel plate
(Original article on page 27)