基础研究

成骨蛋白-1 对髓核抽吸术后兔腰椎间盘组织病理变化的影响

刘航涛[1], 林国叶[1], 李平生[1], 林智军[1], 黄云梅[2], 陈国仙[3], 王万明[4]

(1 南京军区福州总医院第一附属医院骨科 351100 福建省莆田市; 2 福建中西医结合研究院病理室 350108 福州市; 3 福建省莆田市第一医院骨科 351100; 4 南京军区福州总医院骨科 350025 福州市)

【摘要】目的:观察成骨蛋白-1(osteogenic protein-1, OP-1)对髓核抽吸术后兔椎间盘组织病理变化的影响。**方法:**32 只日本大耳白兔,用 21 号针头行 L1/2、L3/4 椎间盘后外侧穿刺,抽吸出部分髓核组织,L3/4 用微量注射器注射 OP-1 25μl 作为实验椎间盘(OP-1 组), L1/2 注射 25μl 生理盐水作为对照椎间盘(Saline 组),L2/3 作为正常对照椎间盘(正常组),术后 2 周、4 周、8 周、12 周分别随机处死 8 只兔,每只取 L1/2、L2/3 和 L3/4 椎间盘,切片,行 Masson 染色,观察椎间盘组织病理学变化情况。**结果:**正常对照组椎间盘髓核组织胶原稀疏、排列有序,细胞可呈岛状分布,纤维环胶原纤维排列整齐,无扭曲及无分层现象,术后 2 周、4 周、8 周、12 周椎间盘组织病理变化不明显。Saline 组椎间盘术后 2 周时髓核部分缺失,胶原排列紊乱,出现大量分布不均匀的无定型颗粒;术后 4 周髓核组织裂隙形成,出现较多的类软骨细胞,胶原纤维扭曲严重;术后 8 周髓核组织广泛裂隙,胶原排列极其紊乱,出现介于类软骨细胞及纤维细胞之间的细胞,较明显的分层裂隙,胶原纤维极度扭曲,部分断裂;术后 12 周凝胶状髓核组织呈现明显纤维化,髓核内出现较多纤维样细胞,类软骨细胞数量明显减少,纤维环出现巨大分层裂隙,伴有部分纤维环断裂。OP-1 组椎间盘术后 2 周髓核胶原排列尚有序,髓核细胞仍较多,纤维环形态接近正常;术后 4 周髓核组织轻微裂隙,胶原排列紊乱,髓核细胞减少,出现类软骨细胞,内层纤维环胶原纤维轻度扭曲;术后 8 周髓核组织较多裂隙,胶原扭曲,类软骨细胞增多,内层纤维环胶原纤维明显扭曲,排列紊乱;术后 12 周时类软骨细胞仍较多,出现少量介于类软骨细胞与纤维细胞之间的细胞,全层纤维环胶原纤维扭曲,排列紊乱,但外层纤维环仍完整,纤维环无巨大裂隙出现。**结论:**OP-1 能延缓后外侧纤维环穿刺髓核抽吸术后兔腰椎间盘髓核细胞及纤维环的退变。

【关键词】成骨蛋白-1;纤维环;髓核;组织病理;髓核抽吸术;兔

doi:10.3969/j.issn.1004-406X.2013.01.12

中图分类号:R361.2 **文献标识码:**A **文章编号:**1004-406X(2013)-01-0054-07

The influence of osteogenic protein-1 on histologic changes of rabbit lumbar disc induced by aspirating the nucleus pulposus/LIU Hangtao, LIN Guoye, LI Pingsheng, et al//Chinese Journal of Spine and Spinal Cord, 2013, 23(1): 54-60

【Abstract】Objectives: To observe the influence of osteogenic protein-1(OP-1) on disc histologic changes induced by aspirating the nucleus pulposus. **Methods:** All the 32 Japanese rabbits' posterior annulus fibrosus of L1/2 and L3/4 disc were stabbed by 21-gauge hypodermic needle, then some nucleus pulposus tissues were aspirated out. 25μl OP-1 was injected into L3/4 disc by using micro-injection syringe as the experimental group (OP-1 group). 25μl normal saline was taken injected into L1/2 as a blank control disc(Saline group). L2/3 was taken as a normal control disc(normal group). At 2, 4, 8 and 12 weeks after operation, each 8 rabbits were executed and the disc tissues of L1/2, L2/3, L3/4 were harvested and sliced rapidly, stained by Masson for observation. **Results:** In the normal intervertebral disc, nucleus pulposus tissue collagen was sparse and lined orderly. The cells were distributed as island. Anulus fibrosus collagen fibers were lined regularly, without distortion and delamination. The intervertebral disc showed no significant pathological change at 2, 4, 8 and 12 weeks after operation. In saline group at 2 weeks after operation, excalation of the nucleus

基金项目:莆田市科技计划项目(编号:2010S09-2)

第一作者简介:男(1982-),主治医师,硕士研究生,研究方向:创伤临床及脊柱外科

电话:(0594)2292361-60633 E-mail:liuhangtao_2000@163.com

通讯作者:王万明 E-mail:wangwm93@126.com

pulposus, collagen disarrangement and a large inhomogeneous amorphous particles could be observed. At 4 weeks after operation, the nucleus pulposus tissue formed cracks. More cartilage cells appeared. Collagen fibers were seriously distorted. At 8 weeks after operation, nucleus pulposus tissue had wide fissure. Collagen was arranged extremely disorder. A few cells appeared just between cartilage-like cells and fibroblasts. Layered fissure was formed. Collagen fibers were extremely distorted, with part of fracture evidenced. Gelatinous nucleus pulposus of disc showed significant fibrosis at 12 weeks after operation. Fibroblasts increased in nucleus pulposus region, and the number of cartilage-like cells decreased. The annulus fibrosus appeared huge layered fissure, with part of fracture evidenced. In OP-1 group at 2 weeks after operation, the nucleus pulposus collagen arrangement was ordered. There were still more nucleus pulposus cells. The shape of anulus fibrosus was close to normal. At 4 weeks after operation, the nucleus pulposus tissue formed little cracks, and collagen disarranged. Nucleus pulposus cells decreased, with cartilage-like cells appeared. The inner annulus collagen fibers were distorted slightly. At 8 weeks after operation, nucleus pulposus tissue had more cracks and distortion of collagen. Cartilage-like cells increased. The inner annulus collagen fibers were distorted significantly. At 12 weeks after operation there were still morecartilage-like cells. A few cells appeared just between cartilage-like cells and fibroblasts. The whole layer of the annulus fibrosus collage fibers were distorted and disorganized, but the outer annulus were intact without huge fissure formation.
Conclusions: OP-1 can retard disc degeneration of rabbit induced by puncturing lateral annulus fibrosus and aspirating the nucleus pulposus.

【Key words】 Osteogenic protein-1; Annulus fibrosus; Ucleus pulposus; Aspiration of nucleus pulposus; Rabbit

【Author's address】 Department of Orthopedics, the First Hospital Affiliated to Fuzhou General Hospital of PLA, Putian, Fujian, 351100, China

椎间盘源性腰痛是临床常见的一种疾病,其根本原因是椎间盘退变。由于椎间盘不含有血管组织,其营养依靠邻近终板的被动扩散,纤维环为软骨性结构,再生能力很弱[1-3],一旦发生椎间盘退变,就很难减缓,最终发展到不可逆的状态。所以,如何干预、延缓椎间盘的退变,是当前骨科面临的一个难题。研究已证实多种生长因子在退变椎间盘(包括人突出椎间盘标本)中的表达发生变化[4]。生长因子是治疗椎间盘退变潜力最大的一类蛋白质,成骨蛋白-1(osteogenic protein-1,OP-1)是目前研究较多的一类生长因子。本课题组成功建立了后外侧纤维环穿刺髓核抽吸致椎间盘退变的动物模型[5],本研究旨在观察 OP-1 对兔腰椎间盘髓核抽吸术后组织病理变化的影响,从而为临床干预椎间盘退变提供一种新的思路。

1 材料与方法

1.1 实验动物及分组

32 只日本大耳白兔 [(南京市江宁区青龙山动物繁殖场,SCXK (苏),2007-0008],3 月龄,体重 2.5~3.0kg,雌雄不限;于术前行腰椎 X 线片检查,排除腰椎畸形病变。所有大耳白兔的 L3/4 椎间盘经刺抽吸出部分髓核组织,用微量注射器注射 OP-1 25μl 作为实验组椎间盘 (OP-1 组),L1/2 椎间盘穿刺抽吸部分髓核组织并注射 25μl 生理盐水作为对照椎间盘 (Saline 组),L2/3 作为正常对照椎间盘(正常组)。

1.2 试剂配制

预先将 OP-1 (以色列 prospec 公司,批号 CYT-333) 按照说明书溶解调试,将 80μg OP-1 加入到无菌生理盐水 1ml 中振动搅拌,配制成 80μg/ml 的浓度备用。

1.3 手术方法

速眠新按 0.15ml/kg 行肌注麻醉,麻醉后背部备皮,使其俯卧于术台上,碘伏充分消毒后覆盖无菌洞巾,按髂嵴准确定位后,于兔 L1/2、L3/4 水平后正中右侧旁开 1cm,分别取长约 3cm 纵行切口,顺肌间隙分离暴露 L2、L4 横突及倒"八"字区域(椎板与横突所构成)[6],钝性分离椎旁软组织,暴露 L1/2、L3/4 右后外侧纤维环,直视下用 10ml 无菌一次性注射器配合 21G 穿刺针,垂直椎间盘侧面行 L1/2、L3/4 椎间盘后外侧穿刺。抽吸出部分髓核,取出的髓核组织呈乳白色胶冻状,每个椎间盘取出的髓核量为 5~8mg。用微量注射器于 L3/4 椎间盘原穿刺处刺入并注入 OP-1 25μl 作为实验椎间盘 (OP-1 组,2μg/椎间盘),L1/2 注入

等量生理盐水作为对照椎间盘(Saline 组),逐层缝合切口,术毕。待动物苏醒后送回动物中心继续饲养。术后肌注庆大霉素 (8 万单位,2 次/日)3d 预防伤口感染。2 只兔于术后第 2 天出现右下肢轻微活动障碍,1 周后恢复,余动物活动如常。

1.4 取材及组织病理观察

于术后 2 周、4 周、8 周、12 周,从耳缘静脉注入空气 20ml 处死动物,剖开背部皮肤,分离背部椎旁肌肉,暴露出腰段脊柱后,分别在 T12/L1 椎间盘和 L5 椎体下段截断取出, 剔除椎骨上附着的肌肉, 修剪每个椎体的附件至仅保留椎体及与之相连的椎间盘, 用钢锯沿着施术椎间盘上下终板外侧小心锯开取出 L1/2、L2/3、L3/4 椎间盘 (保留终板上下各 2mm 厚椎体骨质),迅速放入 10% 中性福尔马林溶液中固定 24h 后, 用快速脱钙液脱钙 48h,正中矢状位切开标本,石蜡包埋、组织切片 (厚 5μm),Masson 染色后进行组织学观察,观察髓核及纤维环的形态改变。

Masson 染色步骤:切片脱蜡至水;Bouin 氏液 56℃ 1h,冲洗至黄色消失;苏木精淡染 5min(除核以外其他组织均不着色),蒸馏水冲洗;浸入丽春红酸性品红液 2~3min; 用 0.2%冰醋酸冲洗 1 次;用 1%磷钼酸分化 3~5min;1%亮绿染 5min,用 0.2%冰醋酸再冲洗 1 次;脱水、透明、封片。染色结果的判断标准:胶原纤维呈绿色或蓝色,细胞质呈红色,细胞核呈深红色或褐色。

2 结果

正常对照组椎间盘纤维环结构完整, 胶原排列规则,结构清晰,与髓核分界清楚;髓核组织稀疏,胶原成稀疏网状,分布均匀,髓核细胞近似呈圆形,可单独分布或 3~5 个细胞成群分布,细胞质充满整个细胞,细胞核小且形状不规则(图 1a~c)。术后 2 周、4 周、8 周和 12 周椎间盘组织病理变化不明显。

髓核组织形态:Saline 组椎间盘术后 2 周时髓核部分缺失,胶原排列紊乱(图 2a);术后 4 周髓核组织裂隙形成,胶原排列紊乱(图 2b);术后 8 周髓核组织广泛裂隙,胶原排列极其紊乱,部分组织玻璃样改变(图 2c);术后 12 周髓核组织粗糙,部分区域呈破絮样,明显纤维化(图 2d)。OP-1 组椎间盘术后 2 周髓核胶原排列尚有序(图 2e);术后 4 周髓核组织轻微裂隙, 胶原排列紊乱 (图 2f);术后 8 周髓核组织较多裂隙,胶原扭曲(图 2g);12 周时髓核组织有较大裂隙形成(图 2h)。

髓核细胞:Saline 组椎间盘术后 2 周髓核组织即出现大量分布不均匀的无定型颗粒(图 3a),4 周时出现较多的类软骨细胞(图 3b),细胞较大、肿胀,髓核细胞明显减少,术后 8 周出现介于类软骨细胞及纤维细胞之间的细胞(图 3c),12 周时髓核内出现较多纤维样细胞, 类软骨细胞数量明显减少(图 3d)。OP-1 组术后 2 周髓核细胞仍较多(图 3e),术后 4 周髓核细胞减少,出现类软骨细胞(图 3f);术后 8 周类软骨细胞增多(图 3g),术后 12 周时类软骨细胞仍保持相对较多的数量,纤维细胞数量较少(图 3h)。

纤维环形态:Saline 组椎间盘术后 2 周即出现部分内层纤维环的扭曲,排列紊乱,部分向髓核

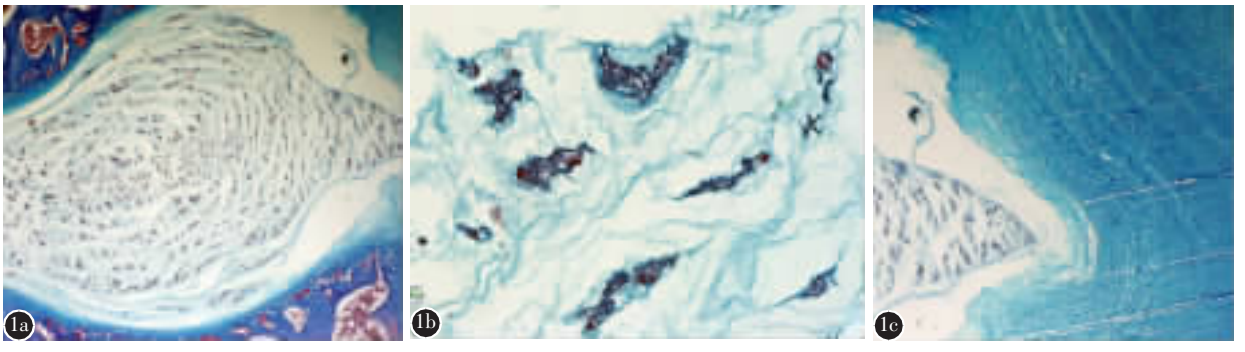

图 1 正常对照组椎间盘(Masson 染色) **a** 髓核组织胶原稀疏,排列有序(×40) **b** 细胞可呈岛状分布(×400) **c** 纤维环胶原纤维排列整齐,无扭曲及分层现象(×40)

Figure 1 The normal intervertebral disc (Masson stain) **a** nucleus pulposus tissue collagen is sparse, arranged in an orderly manner(×40) **b** cells distributed as island(×400) **c** anulus fibrosus collagen fibers arranged regularly, without distortion and delamination(×40)

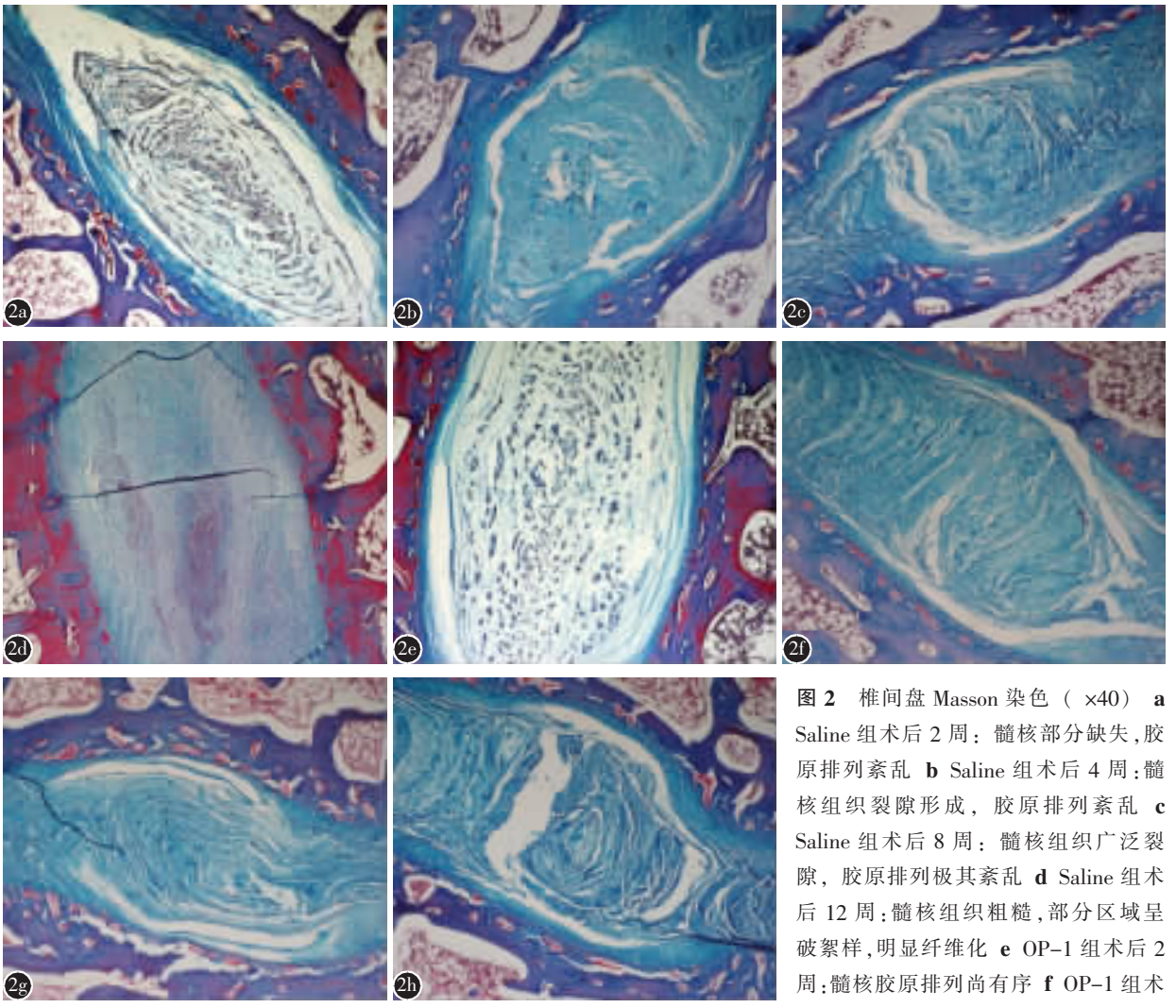

图 2 椎间盘 Masson 染色（×40） **a** Saline 组术后 2 周：髓核部分缺失，胶原排列紊乱 **b** Saline 组术后 4 周：髓核组织裂隙形成，胶原排列紊乱 **c** Saline 组术后 8 周：髓核组织广泛裂隙，胶原排列极其紊乱 **d** Saline 组术后 12 周：髓核组织粗糙，部分区域呈破絮样，明显纤维化 **e** OP-1 组术后 2 周：髓核胶原排列尚有序 **f** OP-1 组术后 4 周：髓核组织轻微裂隙，胶原排列紊乱 **g** OP-1 组术后 8 周：髓核组织较多裂隙，胶原扭曲 **h** OP-1 组术后 12 周：髓核组织有较大裂隙

Figure 2 Masson stain (×40) **a** Group Saline intervertebral disc 2 weeks after operation:with excalation of the nucleus pulposus, collagen disarrangement **b** Group Saline intervertebral disc 4 weeks after operation: the nucleus pulposus tissue formed cracks, collagen disarranged **c** Group Saline intervertebral disc 8 weeks after operation: nucleus pulposus tissue had wide fissures, collagen arranged extremely disorder **d** Group Saline intervertebral disc 12 weeks after operation: the nucleus pulposus was coarse, with part of the region floc-like, significant fibrosis was noted **e** Group OP-1 intervertebral disc 2 weeks after operation:the nucleus pulposus collagen arrangement was ordered **f** Group OP-1 intervertebral disc 4 weeks after operation:the nucleus pulposus tissue formed little cracks, collagen disarranged **g** Group OP-1 intervertebral disc 8 weeks after operation: nucleus pulposus tissue developed more cracks distortion **h** Group OP-1 intervertebral disc 12 weeks after operation: nucleus pulposus tissue had large fissure

内突起(图 4a)；术后 4 周时内层纤维环出现分层裂隙，与髓核分界不清，胶原纤维扭曲严重（图 4b)；术后 8 周时内层及中间纤维环出现分层裂隙加重，部分有轻微的放射状裂隙出现，外层纤维环胶原纤维与髓核分界模糊，胶原纤维极度扭曲，部分断裂(图 4c)；术后 12 周时纤维环出现巨大分层裂隙，伴有部分纤维环断裂(图 4d)。OP-1 组椎间盘纤维环退变出现较迟，较同期 Saline 组椎间盘退变程度轻。OP-1 组椎间盘术后 2 周时尚保持较完整的纤维环形态，内层胶原纤维轻微扭曲(图 4e)；4 周时纤维环形态清晰，内层纤维环胶原纤维轻度扭曲，向内突起，与髓核交界处稍模糊(图 4f)；8 周时外层纤维环形态尚清晰，内层纤维环胶原纤维明显扭曲，排列紊乱，与髓核交界处模

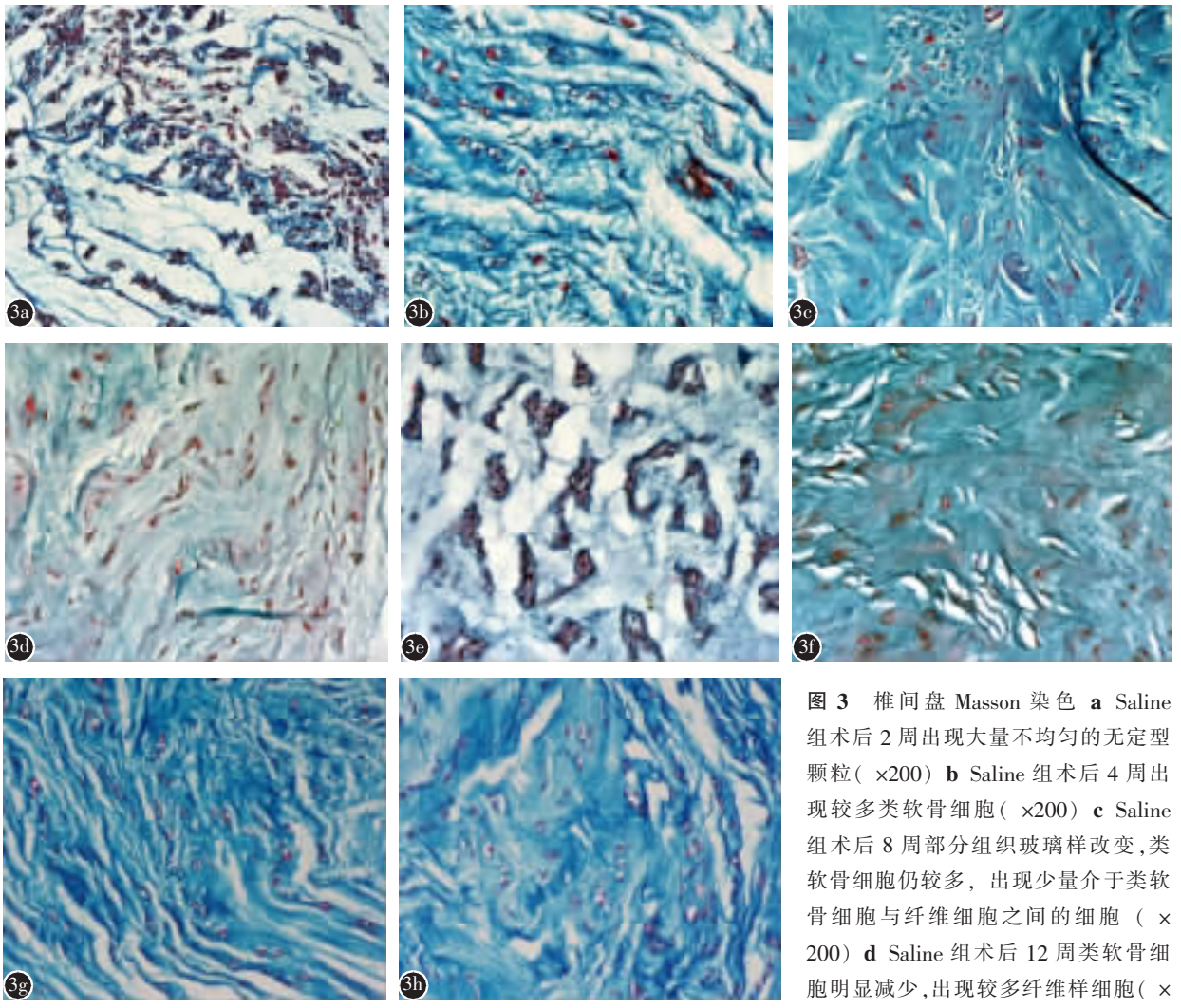

图 3 椎间盘 Masson 染色 **a** Saline 组术后 2 周出现大量不均匀的无定型颗粒（×200）**b** Saline 组术后 4 周出现较多类软骨细胞（×200）**c** Saline 组术后 8 周部分组织玻璃样改变，类软骨细胞仍较多，出现少量介于类软骨细胞与纤维细胞之间的细胞（×200）**d** Saline 组术后 12 周类软骨细胞明显减少，出现较多纤维样细胞（×200）**e** OP-1 组术后 2 周髓核细胞仍较多（×400）**f** OP-1 组术后 4 周髓核细胞减少，出现类软骨细胞（×200）**g** OP-1 组术后 8 周类软骨细胞增多（×200）**h** OP-1 组术后 12 周类软骨细胞仍较多，出现少量介于类软骨细胞与纤维细胞之间的细胞（×200）

Figure 3 Masson stain **a** Group Saline intervertebral disc 2 weeks after operation: a large number of inhomogeneous amorphous particles appeard(×200) **b** Group Saline intervertebral disc 4 weeks after operation: more cartilage cells appeard(×200) **c** Group Saline intervertebral disc 8 weeks after operation: some tissue had glass like change. cartilage-like cells still more, appeared a few cells whose morphous just between cartilage-like cells and fibroblasts(×200) **d** Group Saline intervertebral disc 12 weeks after operation: cartilage-like cells decreased significantly, appeard more fibroblast cells(×200) **e** Group OP-1 intervertebral disc 2 weeks after operation:nucleus pulposus cells still more(×400) **f** Group OP-1 intervertebral disc 4 weeks after operation: nucleus pulposus cells decreased, cartilage-like cells appeard(×200) **g** Group OP-1 intervertebral disc 8 weeks after operation: cartilage-like cells increased(×200) **h** Group OP-1 intervertebral disc 12 weeks after operation: cartilage-like cells still more, a few cells whose morphous were just between cartilage-like cells and fibroblasts appeared(×200)

糊不清（图 4g）；12 周时全层纤维环胶原纤维扭曲，排列紊乱，结构模糊，但外层纤维环仍完整，分层现象轻微，纤维环尚无巨大裂隙出现（图 4h）。

3 讨论

椎间盘退变在组织学水平上可见髓核细胞的减少及基质形态改变，髓核内类软骨细胞的出现及退变后期纤维细胞出现，纤维环胶原纤维排列紊乱及断裂，纤维环出现裂隙和分层现象。基质中的蛋白聚糖进行性减少、纤维环内裂隙形成及胶

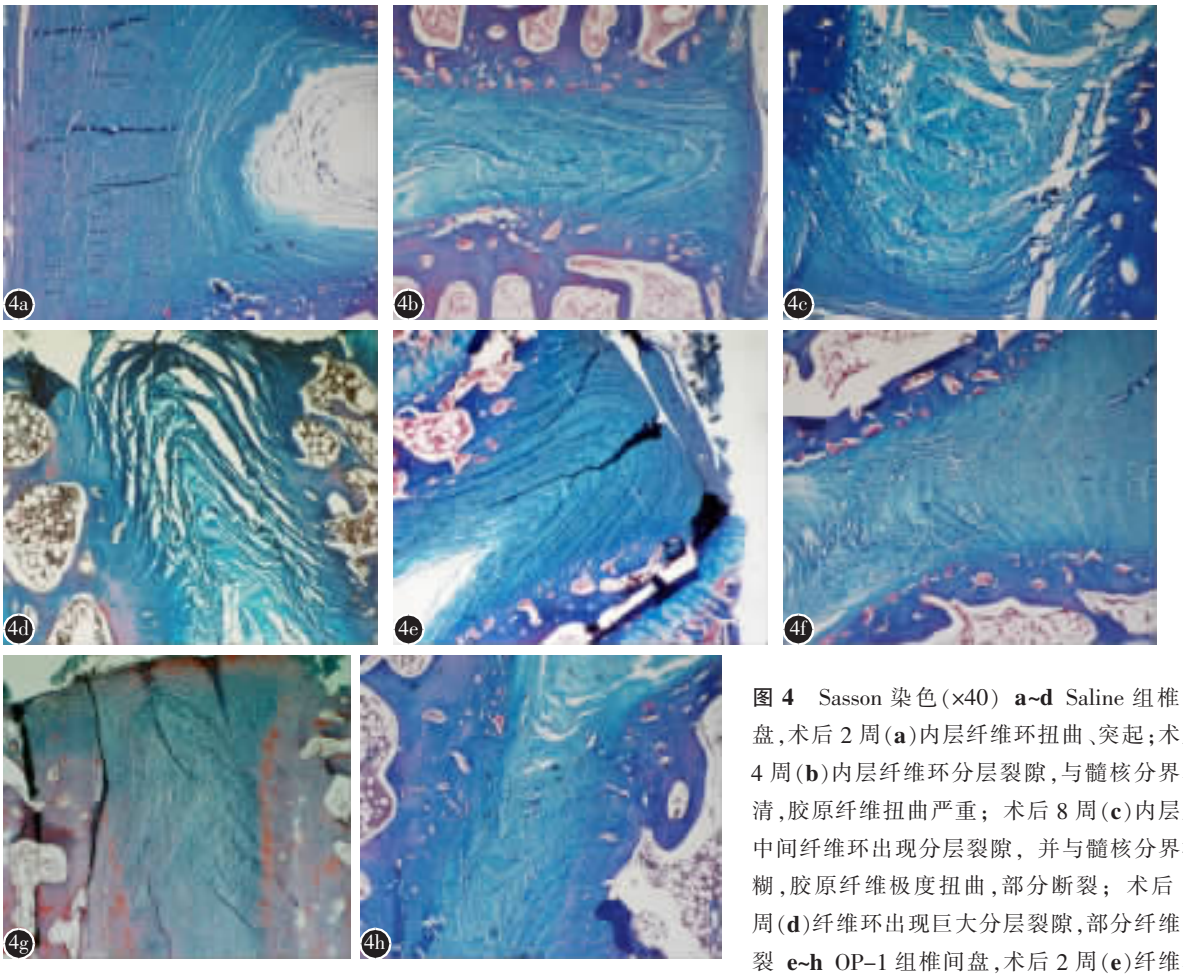

图 4 Sasson 染色(×40) **a~d** Saline 组椎间盘,术后 2 周(**a**)内层纤维环扭曲、突起;术后 4 周(**b**)内层纤维环分层裂隙,与髓核分界不清,胶原纤维扭曲严重; 术后 8 周(**c**)内层及中间纤维环出现分层裂隙, 并与髓核分界模糊,胶原纤维极度扭曲,部分断裂; 术后 12 周(**d**)纤维环出现巨大分层裂隙,部分纤维断裂 **e~h** OP-1 组椎间盘,术后 2 周(**e**)纤维环形态接近正常,内层胶原纤维轻微扭曲; 术后 4 周(**f**)纤维环形态清晰,内层纤维环胶原纤维轻度扭曲,向内突起,与髓核交界处稍模糊;术后 8 周(**g**)外层纤维环形态尚清晰,内层纤维环胶原纤维明显扭曲,排列紊乱,与髓核交界处模糊不清;术后 12 周(**h**)全层纤维环胶原纤维扭曲,排列紊乱,但外层纤维环完整,尚无巨大裂隙出现

Figure 4 Sasson stain(×40) **a–d** Group Saline intervertebral disc **a** 2 weeks after operation: with excalation of the nucleus pulposus, collagen disarrangement **b** 4 weeks after operation: the inner annulus had no clear boundary with nucleus pulposus, layered fracture, collagen fibers seriously distorted **c** 8 weeks after operation: the inner and middle layer anulus fibrosus formed fracture, the boundary was fuzzy with nucleus pulposus. Collagen fibers extremely distorted, partial fracture **d** 12 weeks after operation: the annulus fibrosus had huge layered fissue, with partial of fracture **e–h** Group OP-1 intervertebral disc **e** 2 weeks after operation: the shape of anulus fibrosus is close to normal, the inner layer of collagen fibers slightly distorted **f** 4 weeks after operation: the shape of anulus fibrosus was clear, the inner annulus collagen fibers mildly distorted, protruded inwards. The junction with nucleus pulposus was slightly fuzzy **g** 8 weeks after operation: morphous of the outer anulus fibrosus was clear, the inner annulus collagen fibers were significantly distorted, disordered, and had unclear junction with nucleus pulposus **h** 12 weeks after operation: the whole layer of the annulus fibrosus collage fibers were distorted, and disorganized.But the outer annulus were intact,without huge fissue

原纤维断裂反映出基质重建和胶原组成的变化,胶原、蛋白聚糖的变化以及基质改建等都是椎间盘细胞群变化的结果;因此,椎间盘胶原纤维形态及髓核细胞的数量反映退变的程度[7]。国内对椎间盘退变及修复的实验研究已较深入, 但注入细胞生长因子 OP-1 对腰椎间盘髓核抽吸术后组织病理变化影响的相关研究鲜有报道。

OP-1 即 BMP-7, 属于 TGF-β 超家族成员,最初由 Urist(1964 年)从去矿化的骨基质中发现,与 BMP-5 、BMP-6 为同一亚型[8]。研究表明,OP-1 可促进软骨细胞增殖分化, 刺激正常或老化软骨细胞基质的合成[9]。近年来国内外学者研究发

现,OP-1 对体外培养的椎间盘细胞有刺激细胞代谢及蛋白聚糖和胶原合成的作用，证实 OP-1 对椎间盘细胞体外培养的影响是有利的，能刺激椎间盘细胞的有丝分裂,促进细胞代谢,增加蛋白聚糖及胶原的合成[10,11]。这些研究成果为 OP-1 的椎间盘体内研究打下了基础，并引起了国内外学者对 OP-1 椎间盘体内研究的兴趣。本研究通过注入细胞生长因子 OP-1 到髓核抽吸后的兔椎间盘内,行椎间盘组织病理学检查,观察注入 OP-1 对兔腰椎间盘髓核抽吸术后组织病理变化的影响。

本研究用 Masson 染色椎间盘组织,细胞核染色呈深红色或褐色,细胞质呈红色,胶原纤维呈蓝色或绿色,既可观察髓核细胞变化及胶原排列,也可观察纤维环胶原纤维的变化。Saline 组髓核抽吸术后 8 周椎间盘即呈现明显的退变状态：髓核组织广泛裂隙,胶原排列极其紊乱,部分组织玻璃样改变,类软骨细胞较多,出现少量介于类软骨细胞与纤维细胞之间的细胞，内层及中间纤维环出现分层裂隙,并与髓核分界模糊,胶原纤维极度扭曲,部分断裂。OP-1 组椎间盘髓核组织较 Saline 组在实验同期保持相对完整的形态，虽然也随时间而逐渐出现退变状态,诸如胶原纤维增多、排列紊乱等,12 周时出现较大裂隙，但其退变程度较 Saline 组椎间盘轻;髓核细胞变化较迟,类软骨细胞增殖及存在时间较长,术后 4 周、8 周逐渐占优势,12 周时仍有较多的类软骨细胞，纤维样细胞少,尚无明显的纤维化。OP-1 组椎间盘纤维环退变出现较迟,较同期 Saline 组椎间盘退变程度轻,12 周时虽有内外层纤维环胶原纤维扭曲,排列紊乱,结构模糊,但胶原纤维断裂轻微,纤维环无巨大裂隙出现。说明椎间盘内注射 OP-1 能延缓后外侧纤维环穿刺髓核抽吸术后椎间盘的退变。

OP-1 的作用机理尚不清楚,它能调节细胞生长及细胞外基质的分泌，可能与其他细胞因子相似,能通过内分泌、自分泌和旁分泌三种途径发挥自身生物效应；椎间盘为缺少血供的组织,OP-1 可能是通过自分泌和旁分泌机制调节细胞外基质代谢,从而延缓椎间盘细胞凋亡[12]。实验组椎间盘组织染色未发现髓核内有成骨细胞及骨细胞,即实验期内未发现注射 OP-1 到椎间盘内有任何成骨现象，其延缓髓核抽吸术后椎间盘退变的作用与其成骨作用可能无关，但其长期反复注射是否会在椎间盘内出现成骨现象尚待研究。

4 参考文献

1. Nishida K, Kang JD, Gilbertson LG, et al. 1999 Volvo award in basic science:modulation of the biologic activity of the rabbit intervertebral disc by gene therapy: an in vivo study of adenovirus –mediated transfer of the human transforming growth factor beta1 encoding gene[J]. Spine, 1999, 24(23): 2419–2425.
2. Bradford DS, Cooper KM, Oegema Jr TR. Chymopapain,chemonucleolysis, and nucleus pulposus regeneration [J]. J Bone Joint Surg, 1983, 65(9): 1220–1231.
3. Roughley PJ. Biology of intervertebral disc aging and degeneration: involvement of the extracellular matrix[J]. Spine, 2004, 29(23): 2691–2699.
4. Nerlich AG, Bachmeier BE, Boos N. Expression of fibronectin and TGF–beta 1 mRNA and protein suggest altered regulation of extracellular matrix in degenerated disc tissue [J]. Eur Spine, 2005, 14(1): 17–26.
5. 陈国仙, 王万明, 张志宏, 等. 模拟人后路髓核摘除构建椎间盘退行性变动物模型 [J]. 中国组织工程研究与临床康复, 2009, 13(28): 5433–5437.
6. 刘航涛, 王万明, 庄颜峰, 等. 成骨蛋白–1 对髓核抽吸术后兔腰椎间盘影像学改变的影响[J]. 中国脊柱脊髓杂志, 2010, 20(6): 498–502.
7. Gruber HE, Johnson TL, Leslie K, et al. Autologous intervertebral disc cell implantation: a model using psammomys obesus, the sand rat[J]. Spine, 2002, 27(15): 1626–1633.
8. Ozkaynak E, Rueger DC, Drier EA, et al. OP–1 cDNA encodes an osteogenic protein in the TGF–beta family[J]. EMBO J, 1990, 9(7): 2085–2093.
9. Loeser RF, Pacione CA, Chubinskaya S. The combination of insulin–like growth factor 1 and osteogenic protein 1 promotes increased survival of and matrix synthesis by normal and osteoarthritic human articular chondrocytes[J]. Arthritis Rheum, 2003, 48(8): 2188–2196.
10. Zhang Y, An HS, Song S, et al. Growth factor osteogenic protein–1: differing effects on cells from three distinct zones in the bovine intervertebral disc[J]. Am J Phys Med Rehabil, 2004, 83(7): 515–521.
11. Takegami K, Thonar EJ, An HS, et al. Osteogenic protein–1 enhances matrix replenishment by intervertebral disc cells previously exposed to interleukin–1[J]. Spine, 2002, 27(12): 1324–1325.
12. Masuda K, An HS. Growth factors and the intervertebral disc [J]. Spine J, 2004, 4(6 Suppl): S330–340

（收稿日期:2012–07–20 末次修回日期:2012–10–31）
（英文编审 蒋 欣/贾丹彤）
（本文编辑 李伟霞）