·基础研究·

跑步运动对大鼠不稳定膝关节软骨的影响

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【摘 要】 **目的** 研究跑步运动对大鼠不稳定膝关节软骨的影响。**方法** 将 20 只切断膝关节前交叉韧带的 8 周龄 SD 大鼠按随机数字表法分为自由活动组(对照组)和跑步训练组(实验组),每组 10 只大鼠,2 组再根据处死取材时间分为造模成功 3 周和 6 周各 2 个亚组,每个亚组 5 只大鼠。实验组按 15 m/min 强度进行跑步训练,每天训练 1 h;对照组自由活动,不接受任何干预。于造模成功 3 周和 6 周后采用苏木精-伊红(HE)、甲苯胺蓝及免疫组化染色、透射电镜等方法分别观察和比较 2 组大鼠膝关节的软骨厚度、Mankin评分、蛋白多糖含量、软骨基质Ⅱ型胶原含量及软骨形态结构。**结果** 造模成功 6 周后,2 组大鼠关节软骨的厚度和 Mankin 评分与组内造模成功 3 周后比较,差异均有统计学意义($P<0.05$);造模成功 3 周和 6 周后,实验组软骨厚度分别为(154 ± 13)μm 和(131 ± 15)μm,Mankin 评分分别为(9.93 ± 1.36)分和(11.23 ± 1.57)分,分别与对照组同时间点比较,差异均有统计学意义($P<0.05$)。造模成功 6 周后,2 组大鼠膝关节软骨甲苯胺蓝染色阳性光密度与组内造模成功 3 周后比较,差异有统计学意义($P<0.05$);造模成功 3 周和 6 周后,实验组甲苯胺蓝染色阳性光密度分别与对照组同时间点比较,差异均有统计学意义($P<0.05$)。造模成功 6 周后,2 组大鼠膝关节软骨Ⅱ型胶原纤维免疫组化染色阳性光密度与组内造模成功 3 周后比较,差异有统计学意义($P<0.05$);造模成功 3 周和 6 周后,实验组Ⅱ型胶原纤维免疫组化染色阳性光密度分别与对照组同时间点比较,差异均有统计学意义($P<0.05$)。造模成功 3 周后,实验组透射电镜显示软骨细胞减少,软骨表面有部分断裂;造模成功 6 周后,实验组透射电镜可见软骨表面多处有破损,软骨细胞坏死。**结论** 跑步运动对不稳定膝关节软骨具有破坏效应,可加重关节损伤和软骨基质的破坏,加速软骨细胞的退行性变。

【关键词】 前交叉韧带; 大鼠; 不稳定膝关节; 关节软骨; 运动

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【**Abstract**】 **Objective** To investigate the effect of running exercise on cartilage in rats with an unstable knee joint. **Methods** Twenty 8-week-old Sprague-Dawley rats had their left anterior cruciate ligament cut to model an unstable knee. They were randomly divided into a control group and an experimental group, 10 rats in each group. The control group was given no intervention, while the experimental group accepted running exercise training on na animal treadmill at a velocity of 15 m/min for an hour every day. After 3 and 6 weeks of training, 5 rats were sacrificed and cartilage from the medial condyle of the femur was sampled, decalcificated, embedded and sliced on the sagittal plane. After hematoxylin-eosin staining, toluidine blue staining and immunohistochemical staining, the cartilage thickness, Mankin′ score, the content of matrix collagen and the proteoglycan content of the cartilage matrix were assessed, and the shape and structure of the unstable knee joints were observed under a transmission electron microscope. **Results** The cartilage thicknesses and Mankin′s scores at 6 weeks were significantly different from those at 3 weeks in both groups. In the experimental group the average thickness of cartilage was 154 ± 13 μm at 3 weeks and 131 ± 15 μm at 6 weeks. The corresponding Mankin′s scores were 9.93 ± 1.36 and 11.23 ± 1.57, respectively. Both were significantly different from the control group averages at the same time points. There was also a significant difference in the positive rate of toluidine blue and collagen type Ⅱ staining between the experimental group and the control group at both time points, and in the experimental group between 3 and 6 weeks of training. After 3 weeks of training, fewer chondrocytes were observed under the transmission electron microscope in the experimental group, and fissures were seen on the surface of the cartilages. However, 3 weeks later, quite a few ruptures and a lot of necrotic

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cells could be seen. **Conclusions** Running exercise can damage the cartilage of unstable knee joints and speed up the development of osteoarthritis. Even moderate exercise could aggravate damage to unstable joints and the cartilage matrix, and accelerate chondrocyte degeneration.

【**Key words**】 Anterior cruciate ligament; Rats; Knee joint; Joint cartilage; Exercise; Joint stability

近年来运动疗法已被认为是治疗骨性关节炎(osteoarthritis,OA)的基本疗法之一,运动对关节软骨的作用亦日益受到重视[1]。膝关节 OA 的病理表现中,除以软骨为代表的关节内组织结构的退变外,关节稳定作用的降低也是促使膝关节内组织病变加重的重要环节,而关节动力性稳定结构破坏与否,是 OA 病变发生、发展的重要影响因素[2]。课题组前期的研究表明,适度的跑步运动不仅可以增加大鼠膝关节软骨的表面厚度,还可显著改善关节软骨的代谢[3]。

本研究中,将切断膝关节前交叉韧带的大鼠随 PT98 动物跑台机进行一定强度的跑步运动,通过组织学及形态学的对比,观察跑步运动对大鼠不稳定的膝关节软骨细胞形态以及软骨基质蛋白多糖和Ⅱ型胶原含量的影响。

材料与方法

一、实验动物造模与分组

Sprague-Dawley(SD)雄性大鼠 20 只,无特定病原体(specefic pathogen free,SPF)级,8 周龄,体重 200 ~ 220 g,由同济大学动物实验中心提供。20 只大鼠均采用 30 g/L 的戊巴比妥钠按 30 mg/kg 体重腹腔麻醉,麻醉后剃净左侧膝关节周围毛发、碘伏常规消毒铺巾,取左髌旁内侧切口切开关节囊,打开关节腔,将髌骨向外侧脱位,尽量屈曲膝关节显露前交叉韧带,用小尖刀切断前交叉韧带,采用前抽屉试验确定前交叉韧带断裂,术中不损伤软骨面,彻底止血,将髌骨复位,逐层关闭切口。手术完成后,每日肌肉注射普鲁卡因青霉素 1 次,每次 40 万 U,连续 3 d。

造模成功后,将 20 只大鼠按随机数字表法分自由活动组(对照组)和跑步运动组(实验组),每组 10 只大鼠,2 组再根据取材时间分为造模成功后 3 周和 6 周各 2 个亚组,每个亚组 5 只大鼠。

二、干预方案

造模成功后,各亚组的大鼠即分笼常规饲养,饲养环境温度为 20 ~ 23 ℃,相对湿度 50% ~ 55%,自然光照,自由饮食和活动。实验组 2 个亚组每天参照Bedford等[4]的方法在 PT98 动物跑台机(上海奉贤科技有限公司生产)进行跑步运动,以低运动强度(15 m/min),每日运动 1 h。对照组自由活动,不施加任何干预。

二、标本取材与处理

1. 光镜标本的取材与处理:4 个亚组的大鼠根据取材时间处死,取股骨内髁负重区软骨组织固定、脱钙、逐级脱水、包埋后切片,用于苏木精-伊红(hematoxylin-eosin,HE)、甲苯胺蓝染色和免疫组化检测。

2. 透射电镜标本取材与处理:剪取股骨髁负重区一小块组织,制成 1 mm × 1 mm × 1 mm 大小的软骨标本,固定、脱钙、梯度脱水、包埋后超薄切片,醋酸双氧铀和 70% 硝酸铅双重染色后透射电镜观察。

三、检测指标及方法

1. HE 染色:石蜡切片常规脱蜡,苏木素-1% 伊红染色,脱水,二甲苯透明,中性树胶封片,10 × 40 倍光镜下观察关节软骨关节面、软骨细胞、软骨基质、潮线等情况。按 Mankin′s 评分法对切片进行评分[5],0 ~ 1 分为正常软骨;2 ~ 6 分为早期 OA;7 ~ 10 分为中期 OA;11 ~ 14 分为晚期 OA。将图像输入 Image-Pro plus 6.0 版图像分析系统,测量软骨厚度(从潮线表面至钙化软骨与骨交界处),每组样本测量 5 个标本,每个标本取 5 个视野,取平均值。

2. 甲苯胺蓝染色:树脂包埋组织半膜切片,加热烤干,1% 甲苯胺蓝染色 5 min,水洗后烤干,中性树胶封片。采用 Image-Pro plus 6.0 版图像分析系统,于高倍镜下测定单位面积阳性染色平均光密度值,用以半定量表示细胞基质中蛋白多糖的含量,每组样本测量 5 个标本,每个标本取 5 个视野,取平均值。

3. 免疫组化染色:购Ⅱ型胶原兔抗鼠多克隆抗体免疫组化染色 SP 试剂盒(武汉博士德生物有限公司)。免疫组化实验步骤按照说明书操作:石蜡切片常规脱蜡脱水,加入 1∶100 兔抗大鼠Ⅱ型胶原抗体,4 ℃过夜,滴加生物素标记的羊抗兔二抗,二氨基联苯胺显色,苏木紫复染,二甲苯透明,中性树胶封片。采用 Image-Pro plus 6.0 版图像分析系统,于高倍镜下测定单位面积阳性染色平均光密度值,用以半定量表示细胞基质中Ⅱ型胶原的含量,每组样本测量 5 个标本,每个标本取 5 个视野,最后取平均值。

4. 透射电镜观察:采用日本电子株式会社产的 JEM-1230 型透射电镜,观察软骨细胞超微结构并拍照。

四、统计方法

采用SPSS 13.0版统计学软件对计量资料进行统

计学分析,计量资料以($\bar{x} \pm s$)表示。统计学分析采用配对 t 检验。检验水准 $\alpha = 0.05$,以 $P < 0.05$ 为差异有统计学意义。

结　果

一、2 组大鼠膝关节软骨厚度和 Mankin 评分比较

造模成功 6 周后,2 组大鼠关节软骨的厚度和 Mankin 评分与组内造模成功 3 周后比较,差异均有统计学意义($P < 0.05$);造模成功 3 周和 6 周后,实验组软骨厚度分别为(154 ± 13)μm 和(131 ± 15)μm,Mankin评分分别为(9.93 ± 1.36)分和(11.23 ± 1.57)分,分别与对照组同时间点比较,差异均有统计学意义($P < 0.05$),详见表 1。

二、2 组大鼠膝关节软骨基质蛋白多糖含量的检测比较

造模成功 6 周后,2 组大鼠关节软骨甲苯胺蓝染色阳性光密度与组内造模成功 3 周后比较,差异有统计学意义($P < 0.05$);造模成功 3 周和 6 周后,实验组甲苯胺蓝染色阳性光密度分别为(0.28 ± 0.05)和(0.24 ± 0.07),分别与对照组同时间点比较,差异均有统计学意义($P < 0.05$),详见表 1。造模成功 3 周后,实验组较对照组明显浅淡;造模成功 6 周后,实验组部分区域失染,详见图 1。

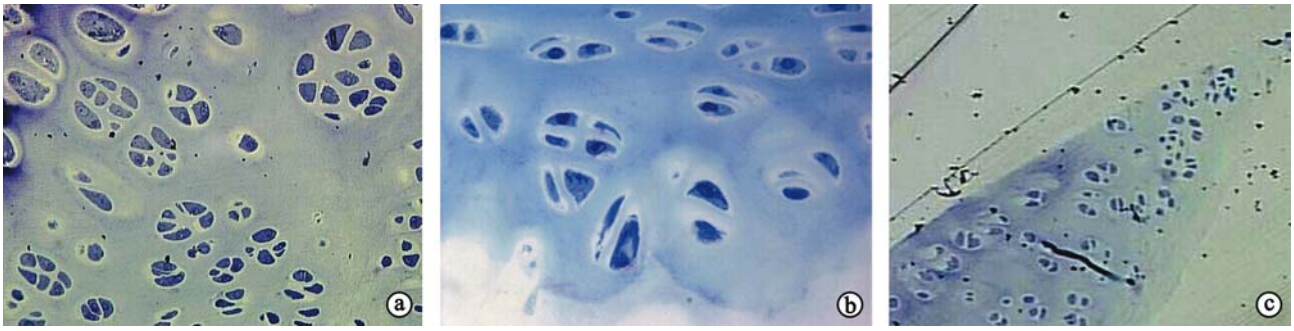
三、2 组大鼠膝关节软骨基质Ⅱ胶原纤维含量的检测比较

造模成功 6 周后,2 组大鼠关节软骨Ⅱ型胶原纤维免疫组化染色阳性光密度与组内造模成功 3 周后比较,差异有统计学意义($P < 0.05$);造模成功 3 周和 6 周后,实验组Ⅱ型胶原纤维免疫组化染色阳性光密度分别为(0.37 ± 0.08)和(0.32 ± 0.03),分别与对照组同时间点比较,差异均有统计学意义($P < 0.05$),详见表 1。造模成功 3 周后,实验组较对照组明显浅淡,而造模成功 6 周后,实验组部分区域更浅染,详见图 2。

表 1　2 组大鼠各项指标检测结果比较(分,$\bar{x} \pm s$)

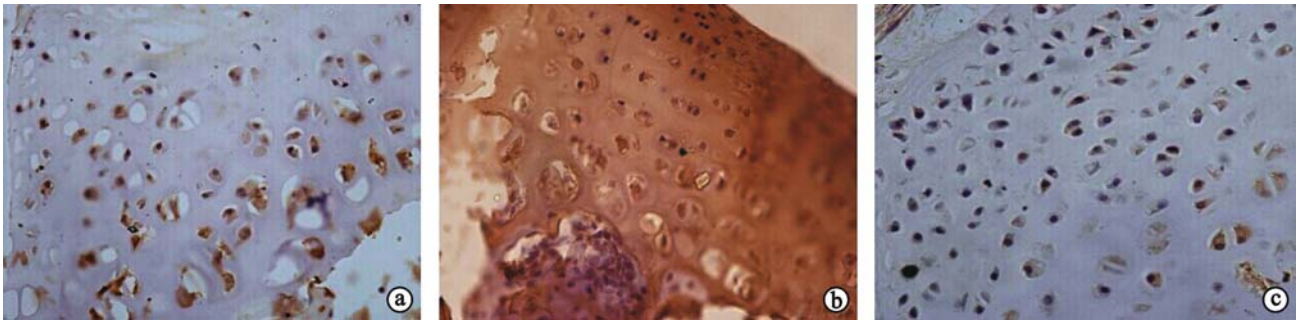
组别	只数	软骨厚度(μm)	Mankin 评分(分)	甲苯胺蓝染色平均光密度	免疫组化染色平均光密度
对照组					
造模成功 3 周后	5	201 ± 21	2.77 ± 0.67	0.38 ± 0.03	0.42 ± 0.07
造模成功 6 周后	5	187 ± 23	4.98 ± 1.09	0.31 ± 0.06	0.39 ± 0.04
实验组					
造模成功 3 周后	5	154 ± 13[a]	9.93 ± 1.36[a]	0.28 ± 0.05[a]	0.37 ± 0.08[a]
造模成功 6 周后	5	131 ± 15[ab]	11.23 ± 1.57[ab]	0.24 ± 0.07[ab]	0.32 ± 0.03[ab]

注:与组内造模成功 3 周后比较,[a]$P < 0.05$;与对照组同时间点比较,[b]$P < 0.05$



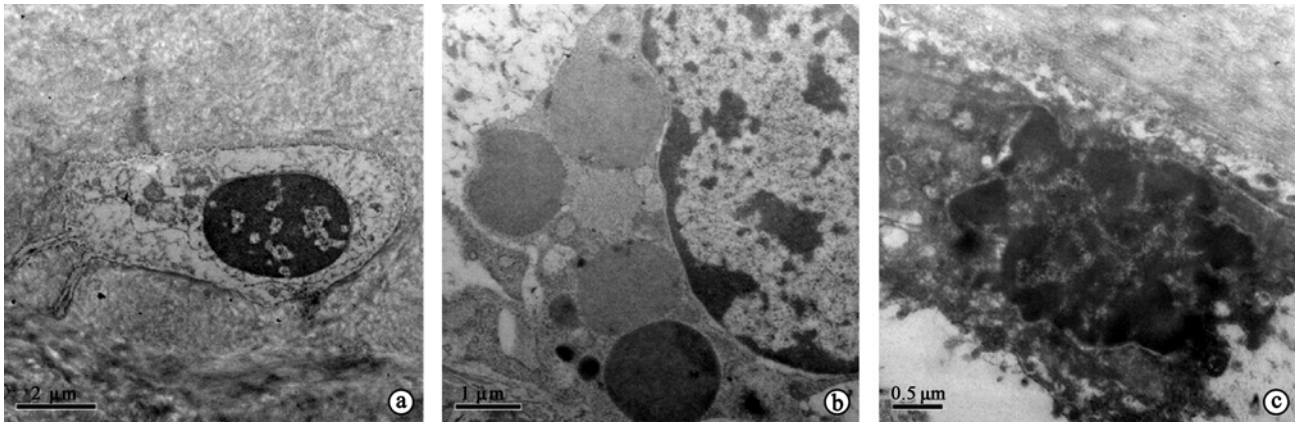
注:a 为造模 3 周后实验组(×400),b 为造模 3 周后对照组(×400),c 为造模 6 周后实验组(×100)

图 1　2 组大鼠膝关节软骨细胞及其基质(甲苯胺蓝染色)



注:a 为造模 3 周后实验组(×200),b 为造模 3 周后对照组(×200),c 为造模 6 周后实验组(×100)

图 2　2 组大鼠关节软骨细胞及基质 II 胶原纤维(免疫组化染色)



注：a 为造模 3 周后对照组，b 为造模 3 周后实验组，c 为造模 6 周后实验组

图 3　2 组大鼠膝关节软骨的超微结构观测

四、2 组大鼠膝关节软骨的超微结构观测比较

造模成功 3 周后，对照组膝关节软骨细胞核膜较清楚，胞浆密度尚均匀，可见内质网、线粒体，实验组膝关节软骨细胞核变形，核膜不连续，可见大量囊泡、脂肪滴；造模成功 6 周后，实验组膝关节软骨细胞轮廓不清晰，细胞核消失，细胞裂解为大量致密的颗粒状物，详见图 3。

讨　论

本研究结果显示，造模成功 3 周和 6 周后，实验组软骨厚度、Mankin 评分、大鼠膝关节软骨甲苯胺蓝染色阳性光密度以及大鼠膝关节软骨Ⅱ型胶原纤维免疫组化染色阳性光密度分别与对照组同时间点比较，差异均有统计学意义（$P<0.05$）。提示跑步运动可能减少不稳定关节软骨蛋白多糖、Ⅱ型胶原纤维的含量，并且随运动持续时间延长减低更为明显，加重不稳定膝关节关节软骨的损伤，加速 OA 的发生及发展。这与 Bramono 等[6] 和 Mandelbaum 等[9] 的结论相一致。还有研究认为，对于结构异常的关节如神经疾病累及的关节和解剖变异、不稳定的关节等，即使正常水平的关节运动也可以引起关节表面损伤和退行性变，发生 OA[8]。Lane 和 Buckwalter[9] 的研究认为，跑步并不增加 OA 的发病风险，但已经有损坏的关节从事剧烈运动可增加 OA 发病率。

关节软骨主要是由软骨细胞和大量的细胞基质构成，关节运动时，软骨基质通过形变达到承受并传导巨大负荷压力，负荷消失后，软骨恢复到原状。正常情况下，关节软骨可始终维持这种功能，即使随着年龄的增加也不至于发生软骨破坏等。

跑步运动是人体的重要运动方式之一，对关节软骨具有重要的影响[10]。Lane 等[11] 对 35 例规律性长跑锻炼者和 38 例不进行锻炼的受试者进行了 5 年的前瞻性调查，结果发现，跑步运动并不增加 OA 的发病风险，因为跑步是一种低撞击性的运动。本课题组前期的研究也发现，跑步运动对于维持关节的功能具有重要的作用，适度的跑步运动可对正常软骨的形态结构有加强和促进作用[3]。对于不稳定的关节，有文献报告，关节不稳定可导致 OA 发生，将犬前十字韧带切断 3 个月后就可成为理想的 OA 动物模型[12]；而 Wright 等[13] 的研究发现，舟月关节、月骨三角骨关节或三角软骨有退行性变者，其关节处的韧带多有撕裂损伤。有研究指出，不稳定的膝关节运动时关节内外结构的运动轨迹、负荷传递可发生改变，而运动可能会引起和加剧关节结构破坏，引起软骨细胞代谢紊乱[14]。

综上所述，跑步运动可能加重不稳定关节软骨基质的破坏，加速软骨细胞的损伤和退行性变，促进 OA 的发生发展，因此，确保膝关节稳定是保证膝关节 OA 运动治疗疗效的基础。

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·外刊撷英·

Platelet rich plasma for pressure ulcers

BACKGROUND AND OBJECTIVE Pressure ulcers are one of the major, secondary complications of spinal cord injury (SCI). These ulcers can be difficult to heal, and can be a source of morbidity and even mortality. As platelet rich plasma (PRP) is considered to be an advanced wound therapy for both chronic and acute wounds, this study evaluated the effect of this treatment on patients with pressure ulcers related to a SCI.

METHODS This prospective study included 25 adult patients with SCI, each with an injury below C-4 and at least two, non-healing pressure ulcers. The larger of the ulcers was chosen for the twice weekly PRP treatment, while the smaller ulcer underwent daily saline dressing. Progress was monitored over five weeks using the Pressure Ulcer Scale for Healing, wound surface area and punch biopsies of the wound margin for histopathology.

RESULTS While scores on the Pressure Ulcer Scale for Healing improved over five weeks in both groups, no significant difference was seen between the two groups. The decrease in wound surface area was significant in the PRP group, but not in the control group. At five weeks, 60% of the PRP group showed well-formed granulation tissue and epithelialization, as compared to 30% in the control group. Also at five weeks, 96% of the ulcers in the PRP group demonstrated improvement, as compared to 60% in the control group.

CONCLUSION This study of patients with spinal cord injury and chronic pressure ulcers found that platelet rich plasma, applied topically to the wound may be superior to standard saline dressings for ulcer healing.

【摘自:Singh R, Rohilla RK, Dhayal RK, et al. Role of local application of autologous platelet rich plasma in the management of pressure ulcers in spinal cord injury patients. Spinal Cord, 2014, 52(11): 809-816.】

Medications for diabetic neuropathy

BACKGROUND AND OBJECTIVE Diabetic neuropathy is a common, long-term complication that can decrease quality of life. While various neuropathic agents are useful for treating this pain, choosing one or the other can be challenging. This systematic review investigated the relative effectiveness of various medications used for the treatment of diabetic neuropathy.

METHODS Databases were reviewed for randomized, controlled trials published between January of 2012 and April of 2014. The trials assessed the efficacy of medications for treating diabetic neuropathy, and compared the medication to placebo or to another medication.

RESULTS Sixty-five, randomized, controlled trials, including 27 medications with 12,632 patients analyzed. By drug class, SNRIs, topical capsaicin, TCAs, and anticonvulsants all resulted in larger and statistically significant reductions in pain, as compared with placebo. Head-to-head trials showed that SNRIs and TCAs reduced pain more than did anticonvulsants and topical capsaicin. Studies that evaluated long-term efficacy found that the aldose reductase inhibitors fidarestat, duloxetine and oxcarbazepine are all more effective than placebo. Indirect and direct comparisons among specific medications revealed greater pain control with carbamazepine, venlafaxine, duloxetine and amitriptyline as compared with placebo.

CONCLUSION This systematic review and meta-analysis of pharmacologic interventions for painful diabetic neuropathy found that carbamazepine, venlafaxine, duloxetine and amitriptyline are significantly better than placebo for controlling pain.

【摘自:Griebeler ML, Morey-Vargas OL, Brito JP, et al. Pharmacologic interventions for painful diabetic neuropathy. Ann Intern Med, 2014, 161(9): 639-649.】