退变性腰椎疾病患者小关节囊中P物质的表达★

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Substance P expression in lumbar facet joint capsule in degenerative lumbar disease patients

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Abstract

BACKGROUND: Previous animal or corpse-based studies show that substance P is increased in degenerative lumbar facet joint bone below cartilage and calcified cartilage nerve fiber in lumbar facet joint-derived low back pain.
OBJECTIVE: To investigate the substance P expression changes in facet joint capsule in patients with degenerative lumbar disease.
METHODS: A total of 18 cases were selected by diagnostic block before operation in degenerative lumbar disease. The test group experienced resection of facet joint capsule, and the control group had no low back pain before fracture. Immunohistochemical method was used to observe the SP immunoreactive fibers in the degenerative facet joints capsule.
RESULTS AND CONCLUSION: In the test group, substance P immunoreactive nerve fibers were observed in 15 cases, and 3 cases had no positive reaction. Substance P immunoreactive nerve fibers distributed in joint capsule and subchondral bone area, and most of them were located in facet capsule. The fibers were also detected in plical tissue of the capsule. They were not all associated with large blood vessels, but some of them ran freely in the stroma. In addition, a few substance P immunoreactive nerve fibers were observed in 5 cases of the control group, and 13 cases were negative. The degenerative change of facet joint capsule is one of the important causes of chronic low back pain. The observation provides morphological basis for low back pain, implying that the substance P immunoreactive fibers might involve in facet degeneration and might be an important source of the lower back pain.

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摘要

背景：以往基于动物实验或尸体的研究发现，腰椎小关节源性腰痛中退变的腰椎小关节软骨下骨和钙化软骨神经纤维中 P 物质增多。
目的：观察退变性腰椎患者中小关节囊中 P 物质的表达变化。
方法：采用术前诊断性封闭方法筛选出 18 例退变性慢性腰痛患者，行小关节囊切除，切除的小关节囊标本作为实验组，选取 18 例骨折前无腰痛的腰椎骨折患者的小关节囊标本为对照组，进行 P 物质免疫组化染色，观察 P 物质免疫组化染色阳性神经纤维的分布情况。
结果与结论：实验组中 15 例可见 P 物质阳性神经纤维，3 例未见阳性反应。P 物质神经纤维在小关节囊内的定位与分布基本一致，关节囊上的 P 物质神经纤维大部分沿血管走行，血管周围阳性神经纤维密度较大，也有一些 P 物质神经不与血管伴行，独立走行于关节囊基质中，另外在小关节软骨下骨区亦有少量独立走行的 P 物质纤维。对照组腰椎小关节囊中 5 例可见少量 P 物质阳性神经纤维，13 例为阴性。由此得出，腰椎小关节囊病理改变与退变性腰痛有明显关系，小关节囊中 P 物质阳性神经纤维可能参与了退变性腰痛疼痛的发生。小关节囊病理改变在小关节源性腰痛发生中起重要作用。
关键词：慢性下腰痛；P 物质；小关节囊；组织构建；骨组织工程
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0 引言

腰椎间盘突出、腰椎小关节退变、背部肌肉及筋膜劳损、骶髂关节炎等被认为是腰痛的主要来源。Ohtori等[1]研究表明小关节退变在腰腿痛的发病中起着重要作用。临床资料显示约15%的年轻患者与约40%的老年患者的慢性腰腿疼是由于腰椎小关节退变所致[2]。

关于腰椎小关节源性腰痛在以往研究结果和推论多基于动物实验或尸体研究[1, 3]，对人体的研究甚少，尤其是结合临床症状和体征探讨小关节源性腰椎疼痛的病理机制研究报道更少。实验通过对诊断性封闭方法筛选的腰椎间盘突出，腰椎管狭窄病人进行手术切除小关节囊，进行病理学及免疫学研究，测定小关节囊中P物质神经纤维的分布，进一步阐明这类患者慢性腰痛的发病机制，为促进慢性腰痛的预防和治疗提供理论依据。

1 对象和方法

设计：配对研究。

时间及地点：实验于2008-03在北京天坛

医院病理诊断中心完成。

对象：病例组为均由腰椎退变引起腰痛超过6个月保守治疗无效，需接受手术处理患者的腰椎小关节囊。纳入标准：腰椎间盘突出症和腰推管狭窄症患者，年龄、性别不限。采用目前诊断小关节源性腰痛的经典方法[4-5]，对患者行术前诊断性封闭：患者俯卧位，C型臂X光机下定位，于腰椎压痛最明显的椎旁小关节行穿刺，局部注射1.0~1.5 mL利多卡因，腰痛明显缓解或消失者成为研究对象，术中切取腰椎小关节囊，留作标本，即腰椎小关节源性腰痛患者腰椎小关节囊，共18例。对照组为骨折前无腰痛病史的腰椎骨折患者腰椎小关节囊标本18例。所有受试者对实验及治疗均知情同意[6]。

试剂及仪器：

试剂及仪器	来源
多聚赖氨酸，P物质，小鼠抗SYN单克隆抗体，羊抗小鼠IgG，二氨基联苯胺	北京中杉生物公司
BX40光学显微镜	日本OLYMPUS
切片机	英国SHANDON

方法：

免疫组化观察：将标本放入40 g/L甲醛中固定20 h左右，0.01 mol/L磷酸盐缓冲液冲洗，50%甲酸脱钙60 h左右，依次放入体积分数70%，80%，90%乙醇、正丁醇脱水，透明，石蜡包埋。将厚4 μm的切片贴附于多聚赖氨酸包被的玻片上，脱蜡，体积分数为3%H_2O_2灭活内源性过氧化物酶10 min，0.01 mol/L磷酸盐缓冲液洗涤3次，小牛血清封闭，0.01 mol/L磷酸盐缓冲液洗涤3次，加入小鼠抗SYN单克隆抗体(1∶40) 4 ℃孵育过夜，37℃复温，0.01 mol/L磷酸盐缓冲液洗涤3次，滴加羊抗小鼠IgG(1∶250)孵育30 min，0.01 mol/L磷酸盐缓冲液洗涤3次，二氨基联苯胺显色。光学显微镜下观察发现有棕黄色物质出现时停止显色反应，苏木精复染，常规脱水、透明、封固。用0.01 mol/L磷酸盐缓冲液代替一抗作为阴性对照。在400倍视野下，随机选取20个视野统计P物质染色阳性的神经纤维的数目。

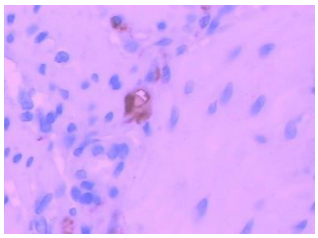
主要观察指标：免疫组化观察退变性腰椎疾患小关节囊中P物质表达。

设计、实施、评估者：实验设计为第一、二作者，实施为第一、三作者，评估为第四作者。实施者及评估者均经过正规培训，采用盲法评估。

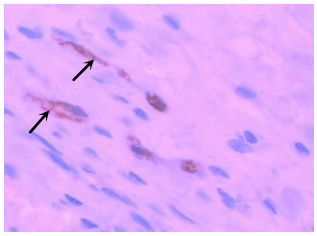
统计学分析：采用SPSS11.0 软件(SPSS公司，美国)进行χ^2检验及t 检验，$P < 0.05$为差异有显著性意义。

2 结果

2.1 腰椎小关节囊P物质表达 腰椎小关节囊P物质阳性神经纤维呈棕色，呈点线状或串珠样。小关节含P物质神经主要存在于关节囊内。关节囊上的P物质神经纤维大部分沿血管走行，血管周围阳性神经纤维密度较大，见图1a，也有一些P物质神经不与血管伴行，独立走行于关节囊滑膜中，见图1b。P物质神经纤维一种呈条索状，蜿蜒走行；另一种表面光滑，笔直分布。



a: Most of substance P was associated with large blood vessels, and positive nerve fiber displayed great intensity around the vessels



b: Nerve fibers are wriggly distributed

Figure 1 Substance P positive nerve fibers in facet joint capsule (Immunohistochemistry, ×400)
图 1 腰椎小关节囊P物质阳性神经纤维(免疫组化，×400)

2.2 各组P物质表达表达差异 见表1，2。

表 1 病例组与对照组小关节囊中P物质阳性神经出现率的比较
Table 1 Incidence of Substance P positive nerve fibers in facet joint capsule between two groups (*n*)

Group	Substance P positive nerve fibers	No substance P positive nerve fibers	Total
Control	5	13	18
Test	15[a]	3[a]	18
Total	20	16	36

[a]*P* <0.01, *vs*. control group

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表2 病例组与对照组P物质阳性神经数量均数比较
Table 2 Mean number of substance P positive nerve fibers between two groups (*n*)

Group	Total number of substance P positive nerve fibers	Number of facet joint capsule	Mean number of substance P positive nerve fibers in facet joint capsule
Control	8	5	1.60
Test	70[a]	15[a]	4.67[a]

[a]*P* < 0.01, *vs.* control group

P物质在退变性腰椎疾病患者中出现较多。

3 讨论

目前公认慢性下腰痛的发病原因为椎间盘源性、小关节源性及骶髂关节源性等[2, 7]。大多数学者研究表明[8-9]，退变是腰痛的首要原因，而腰痛的根本原因来自腰椎间盘的改变：椎间盘本身高度的丢失及维持椎间正常运动的功能丧失，导致椎间盘周围纤维环应力和应变加大，刺激周围神经纤维产生所谓椎间盘源性疼痛[3]。椎间盘内部破裂也可引起腰痛。椎间盘造影可以复制这种疼痛，腰椎间盘源性腰痛的原因占40%以上[10]。而腰椎小关节骨性关节炎是腰椎间盘退变的继发改变：通过大量腰椎MRI和CT扫描结果的对比分析和动物模型的实验研究发现[1, 11-13]；当椎间盘退变较轻时，可不伴有小关节退变；而当椎间盘退变严重时，必定伴有小关节骨性关节炎。

腰椎间盘退变在前，小关节退变在后。这种因果联系得到生物力学上的解释：椎间盘退变后椎间高度丧失是引起小关节骨性关节炎的主要原因。当椎间高度丧失后，小关节将承受异常应力和异常运动。生物力学实验和有限元分析结果显示：随着椎间高度的递减，小关节承受的压应力则显著递增。当关节软骨载荷增加时，一方面软骨的胶原纤维网架遭受破坏，软骨细胞失去保护；另一方面应力增加影响滑液分泌以及软骨组织中物质的交换，使软骨细胞失去营养，出现退行改变且逐渐加重[14]。相应的关节囊充血、水肿、增生、肥大，关节面也可发生软骨破坏，关节突可增生和肥大。由于关节囊的增生和充血水肿，炎性产物刺激支配小关节突的脊神经后支的分支，引起疼痛[15]。

以常规X射线，CT及MRI方法诊断腰痛无法区分腰痛的原因。X射线片可显示小关节密度增高，关节间隙变窄、关节突变尖等。CT可显示小关节间隙的狭窄和关节面破坏程度，小关节的增生及对侧隐窝的影响。MRI除可显示增生的小关节对椎管及神经根管的压迫外，还可显示关节囊的水肿。但这种检查对疼痛原因的判断均为间接的。小关节源性疼痛的确立除有影像学的证据外，诊断性治疗无疑是重要的诊断方法之一。本组病例均是采用了这种经典诊断方法即诊断性封闭[4-5, 13]筛选而出的，可以认为本组病例均合并小关节源性腰痛。

目前国内外的研究已经证实：在动物和人的脊柱小关节囊中存在多种神经肽，包括蛋白基因产物9.5、P物质、降钙素基因相关肽、多巴胺-β-羟化酶、血管活性肠肽、神经肽Y和胆碱乙酰转移酶；这些神经肽可能参与信号传递及疼痛[16]。P物质是发现最早的一种神经肽，由11个氨基酸组成的肽链序列，是初级感觉神经元的调质和初级痛觉传导纤维的传导介质，在神经传递疼痛信息的过程中有重要作用。在慢性腰痛动物模型中，P物质的浓度会发生变化，所以现在一般将其视为疼痛刺激的标志物之一。P物质广泛存在于关节软骨、小关节囊、滑膜细胞层、小血管等组织内，主要对伤害性感受器和炎症有初级的始动作用。同时，P物质参与神经源性神经肽介导的炎症反应，包括引起血管扩张、血浆渗出、诱导肥大细胞释放组织胺等作用。P物质-IR、神经纤维主要存在与腰椎小关节囊中，这些位于关节囊的肽能神经，特别是存在于滑膜皱褶中的肽能神经可能为引起慢性腰痛的神经基础，脊柱发生退变时小关节的应力增加会挤压位于关节软骨间的滑膜皱褶，牵拉小关节囊，从而刺激其中的P物质肽能神经纤维释放P物质引起腰痛发生[13]。

既往对小关节源性腰痛病理机制的研究多基于尸体或动物模型，如Cavanaugh等[13]在退变的腰椎小关节软骨下骨和钙化软骨中观察到P物质阳性的神经纤维，并且提出关节囊的过度张力刺激可能使P物质阳性的神经纤维增多。Yamashita等[17]证明对兔腰椎小关节及其周围组织给予P物质可以引起神经致敏、兴奋性增强，说明P物质可自接使神经末梢致敏或通过扩血管,使肥大细胞释放组织胺等机制间接致敏神经末梢而导致腰背疼痛。

既往对小关节源性腰痛病理机制的研究标本来源于尸体或动物模型，其病理机制却未能在正常人体得到证实，本研究中，实验组和对照组标本均取自人体，腰椎骨折患者小关节囊中P物质表达多为阴性，而小关节源性腰痛患者小关节囊P物质表达多为阳性，P物质阳性神经出现率及数量均存在显著性差异，说明含P物质神经末梢的长入参与了此类患者疼痛的发生发展，从而揭示了小关节源性腰痛患者疼痛发生的病理机制，也进一步证实了既往研究的合理性。当然本研究中样本数量较少，势必影响研究结果的可靠性。

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外国专家修饰的医学英语句型：本刊英文部

中文	修饰前	修饰后
研究基础	Understanding of the progression of cerebral ischemia is essential **to treat** ischemic brain diseases, and the establishment of an animal model of cerebral ischemia is **the basis** for studies of ischemic brain diseases.	An understanding of the progression of cerebral ischemia is essential **for the treatment** of ischemic brain diseases, and the establishment of an animal model of cerebral ischemia is a **requirement** for studies of ischemic brain diseases.
稳定的缺血范围	Moreover, **constant** ischemia range and re-producibility of rats **allows** model establish-ment in cerebral ischemia studies.	Moreover, a **consistent** ischemic range and re-producibility **allows for** the establishment of a rat model in cerebral ischemia studies.
介词短语变成副词	The findings that Ngb decreased cell apoptosis demonstrate that recombinant Ngb introduced in the brain **by stereotaxic technique** can inhibit cell apoptosis in focal cerebral ischemia.	Ngb decreased cellular apoptosis, which demon-strates that **stereotaxically** introduced recombi-nant Ngb into the brain inhibits cell apoptosis **due to** focal cerebral ischemia.
词语的位置与关系最近的放在一起	In conclusion, pCDNA3.1(+)-mediated Ngb expression in focal cerebral ischemia increases Bcl-2 protein expression and inhibit cell apop-tosis **in the ischemic penumbra**	In conclusion, pCDNA3.1(+)-mediated Ngb ex-pression increases Bcl-2 protein expression and inhibits cell apoptosis in the ischemic penumbra **in a model of focal cerebral ischemia**