

DOI:10.3724/SP.J.1008.2012.01301

球囊注射压迫法建立山羊颈脊髓慢性压迫模型

陈智,何平,黄轩,王琨,綦珂,张帆,李凤宁,宋庆鑫,汤译博,侯藏龙,唐沂星,沈洪兴*

第二军医大学长海医院骨科,上海 200433

[摘要] **目的** 验证一种新型颈脊髓慢性压迫动物模型建立方法的可行性。**方法** 选取 18 只崇明山羊,随机分为实验组(15 只)和对照组(3 只)。通过前路手术将球囊压迫装置固定在 C₃ 椎体内,实验组术后每周经皮向注射阀注射 0.1 ml 造影剂,使球囊缓慢膨胀,对颈脊髓产生慢性压迫;对照组放置压迫装置后即刻取出,术后每周仅经皮穿刺但不注射造影剂。每 4 周采用 Tarlov 评分法对动物进行行为学评价,在全麻下进行颈椎 X 线、CT、MRI 检查,并处死 2 只取压迫节段脊髓进行病理学观察。**结果** 对照组各时间点 Tarlov 评分均为 5 分。实验组术后 4 周($n=13$) Tarlov 评分不变;术后 8 周时($n=11$)有 2 只 Tarlov 评分为 4 分,9 只 5 分;术后 12 周时($n=9$)有 3 只 Tarlov 评分为 2 分,4 只 3 分,2 只 4 分。影像学检查示对照组脊髓未见明显异常;实验组球囊压迫系统表现稳定,随着时间推移,脊髓逐渐受压。病理学检查显示对照组未见明显异常。实验组术后 4 周未见明显异常;术后 8 周受压节段脊髓前角内神经元数量减少,胞体周围间隙增大,白质轻度脱髓鞘,部分轴突空泡变性;术后 12 周白质出现片状脱髓鞘区和空泡变性。**结论** 术后实验动物行为学、影像学和组织学检查符合慢性压迫性颈脊髓病特点,说明新型球囊注射压迫系统可以辅助建立稳定、可靠的慢性颈脊髓压迫动物模型。

[关键词] 脊髓压迫症;动物模型;球囊

[中图分类号] R 681.531.1 **[文献标志码]** A **[文章编号]** 0258-879X(2012)12-1301-04

A goat model of chronic cervical spinal cord compression by balloon compression system

CHEN Zhi, HE Ping, HUANG Xuan, WANG Kun, QI Ke, ZHANG Fan, LI Feng-ning, SONG Qing-xin, TANG Yi-bo, HOU Cang-long, TANG Yi-xing, SHEN Hong-xing*

Department of Orthopaedics, Changhai Hospital, Second Military Medical University, Shanghai 200433, China

[Abstract] **Objective** To establish a new animal model of chronic cervical compressive myelopathy and to assess its feasibility. **Methods** Eighteen Chongming goats were divided into two groups: control group ($n=3$) and experimental group ($n=15$). The balloon was placed into the C₃ intervertebral space by anterior approach operation, and the syringe valve was fixed subcutaneously. Contrast agent was injected percutaneously into the valve (0.1 ml/week) to inflate the balloon progressively to produce chronic compression. In the control group, the balloon compression system was placed and immediately removed; percutaneous puncture was performed each week without injecting anything. The Tarlov scores were assessed in each group every four weeks. The goats underwent X-ray, CT and MRI under general anesthesia every four weeks. The spinal cord specimens were pathologically examined at test level at the end of experiment. **Results** The Tarlov scores were 5 (normal) at all time points in the control group. Tarlov scores were not changed in the experimental group four weeks after surgery ($n=13$); at eight weeks after surgery ($n=11$) the Tarlov scores were 4 in 2 goats and 5 in 9 goats; and at twelve weeks after surgery ($n=9$) the Tarlov scores were 2 in 3 goats, 3 in 4 goats and 4 in 2 goats. The balloon compression system was stable in the experimental group. Radiological findings showed that the cervical spinal cord compressed progressively in the experimental group as time went by, and those in the control group underwent no noticeable change. Pathological examination showed neuronatrophy, increased gap around the neurons, mild demyelinated and vacuolar degeneration in the experimental group at eight weeks after surgery, and these changes were deteriorated twelve weeks after surgery. There were no noticeable pathological changes in the control group and four weeks after surgery in the experimental group. **Conclusion** The postoperative behavior, radiological and pathological findings of the animals consist with the character of chronic cervical compressive myelopathy, indicating that the balloon compression system in the present study can be used to establish a reliable and stable animal model of chronic cervical spinal cord compression.

[收稿日期] 2012-08-29 **[接受日期]** 2012-10-29

[基金项目] 上海市浦江人才计划(10PJ1412500). Supported by Shanghai Pujiang Talent Program (10PJ1412500).

[作者简介] 陈智, 博士生. E-mail: mcgrady923@126.com

* 通信作者(Corresponding author). Tel: 021-31161697, E-mail: shenhxgk@126.com

[Key words] spinal cord compression; animal models; balloon

[Acad J Sec Mil Med Univ, 2012, 33(12): 1301-1304]

脊髓型颈椎病等脊髓压迫性损伤是危害中老年人健康的常见疾病,该病起病隐匿、持续进展,其病理机制尚未阐明。由于该病难以依靠临床病例进行系统的病理学和影像学研究,建立合适的动物模型进行深入研究显得尤为重要。脊髓压迫动物模型的建立大致分为螺钉直接压迫模型^[1-2]、可膨胀材料压迫模型^[3-5]、肿瘤压迫模型^[6-7]等,各种模型都有优缺点,学术界尚无共识。近年来对慢性脊髓压迫模型的研究主要集中在啮齿类动物(如大鼠)^[8-9],本实验以山羊为模型动物,采用一种较为简便的球囊注射压迫系统建立了颈脊髓慢性压迫模型,现报告如下。

1 材料和方法

1.1 实验动物与分组 10~12月龄的崇明山羊18只,雌雄不限,体质量40~50 kg(江苏省实验动物中心提供),随机分为实验组(15只)和对照组(3只)。实验组15只在造模后4、8、12周3个节点各处死2只作病理学分析使用,术后12周处死2只后剩余的最后9只供进一步实验分析使用。

1.2 压迫球囊的制备 将安全套(Suretex Limited生产)前端作为球囊,填充在自制的医用内六角聚乙烯螺钉内,后方由硅橡胶封闭,可反复穿刺维持密闭状态,注射5 ml造影剂前后方均无渗漏。螺钉规格:前后径10 mm,外径10 mm,内径8 mm。上述各部分构成整个球囊压迫装置(图1)。

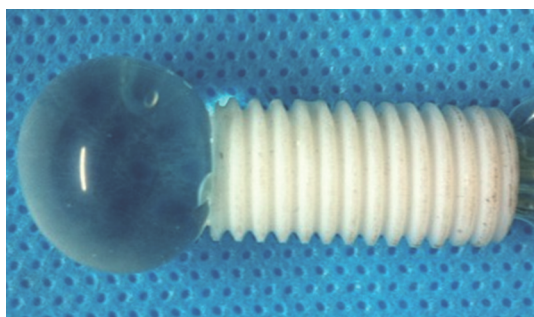


图1 慢性球囊压迫系统

Fig 1 Chronic compression balloon system

1.3 模型制作 实验组动物术前禁食禁水24 h,3%戊巴比妥钠(30 mg/kg)静脉麻醉,气管插管,置于专用手术台仰卧固定,颈后垫枕,维持颈椎中立位。术前定位C₃椎体,左颈前旁正中入路,逐层分离暴露C₃椎体和C_{2/3}、C_{3/4}椎间盘。在C₃前正中用

开口器定位开口,用平头丝攻扩大形成直径1 cm的孔道,后方用神经钩和枪钳进一步暴露,直至显露硬膜囊(图2)。探测椎体深度,将合适尺寸的球囊压迫装置拧入C₃椎体,逐层缝合关闭切口。术前肌内注射青霉素80万单位,术中显露硬膜时静脉注射地塞米松10 mg。术后连续3 d每天肌注青霉素80万单位1次,术后每周经皮注射造影剂碘海醇0.1 ml。对照组动物将球囊压迫装置固定好以后即刻取出,术后每周仅经皮穿刺但不注射造影剂,其余操作过程同实验组。



图2 术中显露C₃水平硬膜囊

Fig 2 Exposure of the dura at the C₃ level

1.4 脊髓运动功能评分 术后每4周采用Tarlov评分^[10]进行脊髓运动功能评分:5分,正常步态,可以跳跃;4分,能跑不能跳;3分,能走不能跑;2分,肢体可以站立,不能走;1分,肢体可以活动但不能站立;0分,肢体完全瘫痪。

1.5 影像学评价 术后每4周在全麻下行颈椎X线、CT、MRI检查。术中出现脑脊液漏、术后X线示球囊窝内造影剂漏至椎管内、麻醉复苏半小时后不能正常站立行走的动物废弃,同时补充实验动物,保证每组实验动物数量。

1.6 病理学检查 对照组动物在实验结束时处死,实验组动物在造模后每4周处死2只,通过手术暴露C₃节段上下长约3 cm的脊髓组织,切取后置入磷酸缓冲液中,常规石蜡包埋,横切片,片厚5 μm,进行HE染色并在光镜下观察。

2 结果

2.1 脊髓运动功能评分结果 实验组动物出现急性脊髓损伤导致死亡1只,及时给予补充。对照组在所有随访时间点神经功能正常(Tarlov评分为5

分)。实验组术后 4 周时所有动物神经功能未见明显异常; 术后 8 周时有 2 只出现后肢运动轻度功能障碍 (Tarlov 评分为 4 分), 此后逐渐加重; 术后 12 周时, 除此前 2 只动物症状加重, 又有 1 只动物出现后肢不能负重, 但蹬腿有力 (Tarlov 评分为 2 分), 4 只动物仅能短距离缓行 (Tarlov 评分为 3 分), 另 2 只出现后肢运动轻度功能障碍 (Tarlov 评分为 4 分)。

2.2 影像学检查结果 术后每 4 周行 X 线、CT、MRI 检测, 结果显示对照组脊髓未见明显异常; 实验组球囊压迫系统表现稳定, 球囊直径从第 1 周的 1.0 mm 增大至第 12 周的 5.9~6.8 mm (平均 6.2 mm), 随着时间推移, 脊髓逐渐受压。术后 12 周末次注射时, X 线检查透视下可见羊颈椎序列好, 注射压迫系统稳定无松脱 (图 3A); CT 示球囊膨胀好, 向椎管内单向压迫且均匀 (图 3B); MRI 示球囊膨胀好, 均匀压迫脊髓 (图 3C、3D)。

2.3 病理学检查结果 HE 染色可见对照组神经元和轴突结构正常 (图 4A、4D)。实验组术后 4 周时表现与对照组相同; 术后 8 周时, 镜下见受压节段脊髓前角内神经元胞体缩小, 胞体周围间隙增大, 神经元未见明显减少, 白质轻度脱髓鞘, 部分轴突空泡变

性 (图 4B、4E); 术后 12 周时, 神经元变性明显, 部分神经元坏死, 神经细胞核固缩, 白质弥漫性脱髓鞘改变, 轴突空泡变性明显 (图 4C、4F)。



图 3 末次注射后影像学结果

Fig 3 Images after the final injection

A: Percutaneous contrast agent injection under X-ray; B: CT axial view; C: MRI sagittal view; D: MRI axial view

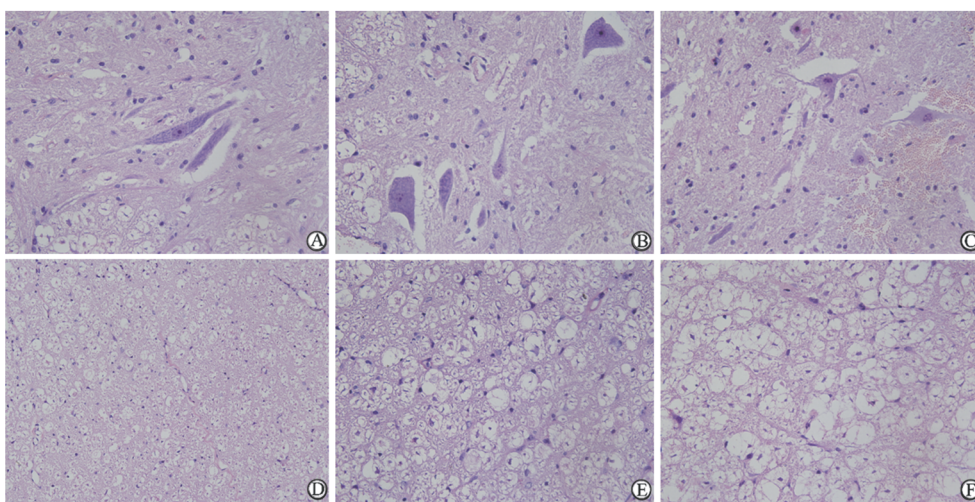


图 4 脊髓慢性压迫神经病理学改变

Fig 4 Spinal pathology in chronic cervical spinal cord compression (HE staining)

A: Normal neurons in the control group; B: Neuronal degeneration and neuronatrophy 8 weeks after operation in the experimental group; C: Neuron death 12 weeks after operation in the experimental group; D: Normal white matter and axon in the control group; E: Mild demyelination and vacuolar degeneration 8 weeks after operation in the experimental group; F: Moderate demyelination and vacuolar degeneration 12 weeks after operation in the experimental group. Original magnification: $\times 100$

3 讨论

建立脊髓压迫模型的方法包括肿瘤细胞的移

植、螺钉置入后渐进性拧入、可膨胀材料的置入以及血管结扎外加螺钉压迫等。Manabe 等^[6]将纤维肉瘤细胞移植到鼠的脊髓硬膜外, 结果该肿瘤细胞生

长迅速,13~18 d即造成了鼠的瘫痪。Ikeda等^[7]研究了癌细胞植入后对兔脊髓表面血供的影响,结果发现在白质中出现了水肿、坏死和血肿,灰质中改变程度较小,瘫痪在术后18 d出现。这些肿瘤模型应用了转移瘤细胞,外形极不规则,生长速度和范围可控性较差;脊髓损伤发展过于迅速,不能很好地模拟慢性脊髓损伤过程;除此之外,肿瘤细胞本身也会侵蚀正常脊髓组织。Kim等^[3]将芳香族聚合物植入鼠的颈椎管内硬膜外,第17周时发现了运动神经元损伤。Kasahara等^[4]将可膨胀材料植入鼠胸椎椎管内硬膜外,在术后6周和9周时发现神经元丢失,但术后15周时仍未发现四肢瘫痪症状。

为了模拟临床上人类脊髓慢性压迫过程,Shinomiya等^[1]采用猫作为模型动物,将螺钉拧入椎体,早期的轻度压迫并未出现瘫痪表现,但数月后的迟发性的脊髓慢性压迫损伤导致了猫的步态异常;组织学研究提示灰质中出现变化,神经元持续减少。al-Mefty等^[2]在犬的前路椎体上放置特氟龙螺钉,渐渐拧入直至椎管侵占率达35%或出现神经电生理检测的异常表现;60周后,犬出现了迟发性脊髓病,合并步态异常;组织学观察发现,运动神经元和血管病变产生,白质中也出现了脱髓鞘等病理改变。Klironomos等^[5]采用芳香族聚合物放置在兔C₆椎板下方,后方渐进性压迫脊髓,术后20周造成颈椎脊髓压迫损伤,较好地模拟了临床上人脊髓型颈椎病的病理改变过程。本实验采用经颈前路手术在C₃椎体内置入注射压迫系统,术后定期少量多次经皮注射造影剂,致压球囊缓慢膨胀压迫颈脊髓,以模拟慢性压迫性颈脊髓病的发病情况。综合本研究动物行为学评价、影像学和组织学检查来评估脊髓慢性压迫损伤过程,结果发现实验组术后4周所有动物神经功能未见明显异常;至术后8周2只动物出现后肢运动轻度功能障碍,此后逐渐加重;术后12周2只动物症状加重,1只出现后肢不能负重,但蹬腿有力,4只动物仅能短距离缓行。推测在早期压迫过程中,由于椎管内存在一定缓冲空间,脊髓可以适度向后方避让,因此到12周时才出现较为明显的神经功能障碍。

本研究采用医用聚乙烯螺钉和球囊系统,优点如下:(1)相对于金属螺钉而言质地较软,避免了与硬膜表面的直接接触损伤,有一定的避让空间,更容易模拟慢性脊髓损伤的病理过程;且可以进行MRI检测,降低了伪影的干扰。(2)压迫程度也能通过注入液体的体积较好地控制。(3)造模手术方法简单,一般可以控制在30 min以内。(4)通过球囊间隔注射造影剂造模避免了反复多次切开手术,避免了体

内留置导管等,降低了手术操作难度,而且降低了感染的风险。(5)造模方式采用脊髓腹侧的压迫方式,较好地模拟了临床上脊髓腹侧慢性受压。上述这些优点为慢性脊髓损伤治疗方面的相关研究提供了方便。但本实验也存在一些不足,如样本量较小,观测时间(12周)不够长,注射的频率、剂量单一(每周0.1 ml)等,尚需加大样本量,调整注射频率和剂量,延长观察时间,进一步探索模型的改良设计,对脊髓慢性压迫损伤的后续进展和转归做进一步观察,更好地实现该模型的科研应用价值。

4 利益冲突

所有作者声明本文不涉及任何利益冲突。

[参考文献]

- [1] Shinomiya K, Mutoh N, Furuya K. Study of experimental cervical spondylotic myelopathy[J]. *Spine (Phila Pa 1976)*, 1992, 17 (10 Suppl): S383-S387.
- [2] al-Mefty O, Harkey H L, Marawi I, Haines D E, Peeler D F, Wilner H I, et al. Experimental chronic compressive cervical myelopathy[J]. *J Neurosurg*, 1993, 79: 550-561.
- [3] Kim P, Haisa T, Kawamoto T, Kirino T, Wakai S. Delayed myelopathy induced by chronic compression in the rat spinal cord [J]. *Ann Neurol*, 2004, 55: 503-511.
- [4] Kasahara K, Nakagawa T, Kubota T. Neuronal loss and expression of neurotrophic factors in a model of rat chronic compressive spinal cord injury [J]. *Spine (Phila Pa 1976)*, 2006, 31: 2059-2066.
- [5] Klironomos G, Karadimas S, Mavrakis A, Mirilas P, Savvas I, Papadaki E, et al. New experimental rabbit animal model for cervical spondylotic myelopathy [J]. *Spinal Cord*, 2011, 49: 1097-1102.
- [6] Manabe S, Tanaka H, Higo Y, Park P, Ohno T, Tateishi A. Experimental analysis of spinal cord compressed by spinal metastasis [J]. *Spine (Phila Pa 1976)*, 1989, 14: 1308-1315.
- [7] Ikeda H, Ushio Y, Hayakawa T, Mogami H. Edema and circulatory disturbance in the spinal cord compressed by epidural neoplasms in rabbits [J]. *J Neurosurg*, 1980, 52: 203-209.
- [8] Xu P, Gong W M, Li Y, Zhang T, Zhang K, Yin D Z, et al. Destructive pathological changes in the rat spinal cord due to chronic mechanical compression. Laboratory investigation [J]. *J Neurosurg Spine*, 2008, 8: 279-285.
- [9] Lee J, Satkunendrarajah K, Fehlings M G. Development and characterization of a novel rat model of cervical spondylotic myelopathy: the impact of chronic cord compression on clinical, neuroanatomical, and neurophysiological outcomes [J]. *J Neurotrauma*, 2012, 29: 1012-1027.
- [10] Tarlov I M, Klinger H, Vitale S. Spinal cord compression studies. I. Experimental techniques to produce acute and gradual compression [J]. *AMA Arch Neurol Psychiatry*, 1953, 70: 813-819.