·论著·

嗅鞘细胞髓内移植修复完全性晚期脊髓损伤手术多中心比较

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摘要 **目的:**比较嗅鞘细胞(OECs)髓内移植修复脊髓损伤(SCI)手术多个临床中心结果,探讨适合临床要求的标准化移植手术。**方法:**汇总有代表性的临床治疗中心的研究结果,分析其移植细胞浓度、细胞总量、切口选择、移植部位、移植点数目、移植器械的优缺点、术后管理等方面,对比这些不同的参数对临床结果的可能影响。**结果:**共纳入4项临床研究,均是采用脊髓髓内实质内移植手术方式治疗晚期完全性SCI,包含中国中心2个,澳大利亚中心1个,欧洲中心1个,进行对比。细胞植入脊髓实质对晚期完全性SCI的功能恢复有一定效果。符合临床实际的简洁手术可能比精准的复杂操作手术更有优势。单位体积内过大移植容量,过长手术时间、过多细胞移植穿刺注射点可能是导致手术效果不佳的重要原因,移植术后需要足够的康复训练。**结论:**细胞植入脊髓实质对晚期完全性SCI患者神经功能恢复有效;简洁的手术可能比精准复杂注射操作更适合临床,但不适当的操作程序会导致无效的结果;移植术后需要足够的康复训练。

关键词 嗅鞘细胞;脊髓内移植;神经修复;完全性晚期脊髓损伤;手术;多中心比较

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Multi–Center Comparison of Olfactory Ensheathing Cell Intramedullary Transplantation for Treatment of Complete Chronic Spinal Cord Injury *CHEN Lin[1], HE Xi-jing[2], HUANG Hong-yun[3], XIAO Kai[1], CUI Zhi-qiang[1], ZHANG Yu-qi[1]. 1. Department of Neurosurgery and Department of Neurorestoratology, Yuquan Hospital, Tsinghua University, Beijing 100040, China; 2. Department of Orthopaedics, Second Affiliated Hospital of Xi'an Jiaotong University, Xian 710004, China; 3. Institute of Neurorestoratology, Third Medical Center, General Hospital of PLA, Beijing 100039, China*

Abstract **Objective:** To compare the results of intramedullary transplantation of olfactory ensheathing cells (OECs) in the repair of spinal cord injury (SCI) in patients at multiple clinical centers, and to explore the standardized transplantation procedures that suit clinical needs. **Methods:** The results of representative clinical centers were collected and analyzed. Parameters including the concentration of cells, total number of cells, choice of incision, site of transplantation, number of transplantation sites, advantages and disadvantages of transplantation equipment, and postoperative management were compared to clarify impact on clinical outcomes. **Results:** Four clinical cases were compared in this study: two Chinese centers, one Australian center, and one European center; all cases employed intraspinal intramedullary intraspinal transplantation for the treatment of chronic complete SCI. Cell implantation into the spinal cord parenchyma had certain effect on recovery in chronic complete SCI. Concise surgery appeared to be more suitable for clinical application than precise and complex injection procedures. An excessively large total volume of injection, long procedure time, and large number of cell transplant injection points are possible reasons for suboptimal results of surgery. Sufficient rehabilitation training after transplantation is necessary. **Conclusion:** Cell implantation into the spinal cord parenchyma is effective for recovery of neurological function in chronic complete SCI patients. Concise surgery appears to be more suitable for clinical application than precise and complex injection procedures, but improper procedures may lead to ineffective results. Sufficient rehabilitation training post-surgery is necessary for the integration of motor recovery.

Key words olfactory ensheathing cells; intraspinal transplantation; neurorestoration; chronic complete spinal cord injury; surgery; multicenter comparison

创伤性脊髓损伤(traumatic spinal cord injury,tSCI),特别是晚期完全性脊髓损伤仍然缺乏有效的治疗策略。神经修复医学的策略是开发从实验室到临床的一整套新疗法,是有希望的新疗法[1-3]。目前,在细胞疗法领域已经取得了显著的进展,多个国家的多个临床中心已经开展了SCI细胞移植(包括神经干细胞、少突胶质细胞、雪旺细胞、嗅

鞘细胞、间充质细胞等）的临床应用[4-6]。嗅鞘细胞（olfactory ensheathing cells，OECs）是一种特殊类型胶质细胞，兼有星形胶质细胞、少突胶质细胞和施万细胞等的多重功能，其神经修复能力优于现在已知的其他类型胶质细胞。它能诱导和引导轴突生长，帮助再生嗅神经纤维穿越神经胶质瘢痕和周围神经与中枢神经之间的屏障，促进嗅神经或其它受损神经再生、髓鞘化修复等结构和功能修复，有较大的应用前景[7-9]。脊髓髓内实质内移植OECs，是治疗tSCI首选的手术方案，但已经报道的临床研究结果并不一致[10,11]。本文收集汇总相关资料并进行分析，以更好推动脊髓实质性细胞移植操作的规范化。

1 资料与方法

通过Pubmed和中国知网数据库，联机检索最近20年内有代表性的临床中心的有关OECs临床移植治疗晚期SCI的研究论文，语言限于中文和English。同时通过国际会议面谈和发送电子邮件等方式与相关研究者充分交流沟通，选择其中最有代表性的研究团队。详细汇总各临床流程参数，包括切口大小、细胞浓度、细胞总量、移植部位、移植点数目、移植器械的设计与选用、术后管理，分析对比其对临床结果的可能影响。

2 结果

目前全球多个国家开展相关临床研究（见表1）[12-14]，共纳入4项临床研究，均是采用脊髓髓内实质内移植手术方式治疗晚期完全性SCI，包含中国中心2个，澳大利亚中心1个，欧洲中心1个，见表2[7,10,15-17]。入选的各项研究的OECs脊髓髓内移植手术参数、并发症、临床疗效等对比结果见表2。结果显示，大部分移植操作病例，术后均获得感觉、运动等脊髓神经功能的不同程度的改善，3年以上的长期随访也证实了这一点；如果注射点过多、注射容积过大，会导致脊髓医源性副损伤和无效病例，例如澳大利亚团队的2个受试患者脊髓内分别注射了599.5 μL（545个注射点，24,000,000个细胞）、693 μL（630个注射点，28,000,000个细胞）[16,17]。

3 讨论

OECs的特性使其能促进SCI受损神经修复，包括神经可塑性改变、轴突髓鞘修复与再生、无功能神经激活、神经侧芽和环路重建、与神经胶质瘢痕相互作用促进血管生成[3,4]。尽管鞘内脑脊液注射是近乎无创，但多数研究者仍推荐首选通过医用注射细针或玻璃毛细管注入微升级细胞悬液的方式，将OECs直接移植进入脊髓损伤部位或与之相邻节段区域实质内[8]。髓内似乎是细胞移植的最佳位置，移植的细胞可以直接与宿主环境交互作用，建立传入和传出轴突的通道，髓鞘化修复轴突，替代丢失的细胞[9,18]。

但值得注意的是，脊髓实质内注射有以下风险：由针刺引起的额外伤害，注射过程中的脊髓在脑脊液内浮动，滴注的细胞团占位压迫周围组织，在脊髓实质内产生压力梯度和水动力分离，并可能导致脊髓缺血。故细胞移植部位、细胞浓度、细胞总量、移植器械和移植手术技巧等，对安全性和疗效非常重要[19]。

表1 全球主要OECs移植治疗晚期完全性SCI临床研究一览

作者	国别	年份/年	病例数/例	细胞类型	移植途径
Huang H, et al.	China	2003	171	OEC	cord parenchyma
Rabinovich SS, et al.	Russia	2003	15	hemopoietic tissues+OEC	subarachnoidally
Féron F, et al.	Australia	2005	3	OEC	cord parenchyma
Guest J, et al.	United states	2006	1	OEC	cord parenchyma
Lima C, et al.	Portugal	2006	7	olfactory mucosa	cord parenchyma
Huang H, et al.	China	2006	222	OEC	cord parenchyma
Mackay-Sim A, et al.	Australia	2008	6	OEC	cord parenchyma
Lima C, et al.	Portugal	2010	20	olfactory mucosal autografts	cord parenchyma
Zheng ZC, et al.	China	2010	213	OEC	cord parenchyma
Huang H, et al.	China	2012	108	OEC	cord parenchyma
Wu J, et al.	China	2012	11	OEC	cord parenchyma
王栋, 等	中国	2012	24	OEC	cord parenchyma
Rao Y, et al.	China	2013	8	OEC	cord parenchyma
Tabakow P, et al.	Poland+UK	2013	6	autologous mucosal olfactoryensheathing cells and olfactory nerve fibroblasts	cord parenchyma

表2 各中心临床OECs脊髓髓内移植手术参数与临床疗效对比

作者	病例数	切口大小/cm	移植部位	细胞浓度/(个/μL)	细胞总量	移植器械	平均手术时间/h	术后管理	随访时间	并发症	临床疗效
黄红云,等	171	4	损伤区与正常上下交界区,2~4个注射点	10,000	50万/50μL	4.5# syringe needle(有药监局批号)	1.5	术后康复	2~8周	无严重并发症	改善
王栋, 等	24	4~6	脊髓损伤区远、近端各0.5 cm处、两侧脊髓灰质,4个注射点	10,000	50万/40μl	5号头皮针(有药监局批号)	1.7 (1.5~2.5)	术后康复	3.2 (0.5~5.2)年	无	10例全瘫患者中9例术后损伤感觉平面下降1~2个脊髓节段,运动未见变化,1例患者损伤平面未见变化,痉挛症状在术后明显减轻。
Féron F, et al.	3	4~6	1μL/次, 脊髓损伤区,远、近端各1个椎体节段。3例注射点数目分别为:270,545和630	80,000	病例1: 297 μL (12,000,000) 病例2: 599.5 μL (24,000,000) 病例3: 693 μL(28,000,000)	自制微量注射器械(无药监局批号)	4	术后康复	3年	无	在1个移植接受者(1号患者)中,轻触和针刺觉在两侧,躯体前后都有改善,下降超过3个皮节
Tabakow P, et al.	3	6~8	脊髓损伤区,远、近端各1个椎体节段,0.5μL/次	病例1: 20部位,120次注射;病例2: 40部位, 128次注射;病例3: 46部位,212次注射。	30,000~200,000 病例1: 60 μL (1,800,000); 病例2: 64 μL (1,920,000); 病例3: 106 μL (21,200,000)	微量注射器械(无药监局批号)	9~11	术后康复	1年	远期无。近期发烧(T1, T2, T3)、尿路感染(T1, T2)、轻度贫血(T1, T2)、需要输血的贫血(T3)、全身性低血压(T3)、压疮溃疡(T1)、肌皮神经暂时性失用(T1)。	前2名手术患者ASIA A to ASIA C and ASIA B。弥散张量成像显示患者脊髓部分白质束恢复了连续性。第3名患者保持ASIA A,但运动和感觉功能在损伤程度下降1个节段。神经生理学检查显示手术治疗患者的脊髓传导和下肢肌肉活动有所改善

表2 各中心临床OECs脊髓髓内移植手术参数与临床疗效对比(续)

作者	病例数	切口大小/cm	移植部位	细胞浓度/(个/μL)	细胞总量	移植器械	平均手术时间/h	术后管理	随访时间	并发症	临床疗效
黄红云,等	108例(康复组79例;康复训练不足组29例)	2~3锁孔手术	损伤区与正常上下交界区,2~4个注射点	10,000	50万/50μL	4.5# syringe needle(有药监局批号)	1	术后康复	(3.47±1.12)年	无	平均运动评分、轻度触觉分、针刺分、IANR-SCIFRS均上升。足量康复训练对运动评分改善有显著影响。12.96%ASIA A改善至ASIA B;16.67%ASIA A改善至ASIA C,8.33%提高了步行能力;14.29%性功能得到改善。29例肌电图改善;28例PVSEP测试改善。

理论上讲,较多点的微量注射,可能促进移植细胞与宿主融合。在实验室研究中,可以长时间进行非常细致的多点小容量注射。但人类的脊髓,不同于各种动物(包括灵长类),其脊髓运动或浮动范围很大,受呼吸、脉搏、脑脊液流动的影响明显。为减少针头损伤,更少的手持注射可能是目前最佳的选择(澳大利亚研究中患者2#和3#的失败提供了佐证)。尤其在颈椎和T_{11}-L_1节段及不完全损伤的SCI,应尽力避免[16,17]。

切口的大小一般应保证显露需要移植的脊髓节段,但黄红云团队采用的锁孔手术,可用于无粘连和囊肿处理的患者,平均手术时间缩短至1 h。相对于欧洲的长达9~11 h的手术时间[15],减小切口,可大大减少可能的麻醉及手术相关并发症,也可缩短手术后的恢复时间。特别应注意,从实验室到移植结束的黄金时间为2 h[11]。

减少注射总容积,适当提高细胞悬液的浓度是改进的一个方向。但临床注射器的针头的内径有限,而过高的细胞浓度可能增加细胞通过针头的阻力,还可能对细胞造成挤压损害。所以,适宜的细胞浓度、细胞总容积及适当的注射点数目,值得深入研究。

本文也显示,足量科学的康复训练,对于患者运动功能的恢复至关重要。单纯细胞移植,如不联合康复训练,无法激活动神经肌肉活动的再支配[10]。

4 结论

细胞植入脊髓实质对晚期完全性SCI的功能恢复有一定效果。符合临床实际的简洁手术可能比精准的复杂操作手术更有优势:节省手术时间、减少医源性副损伤、减少相关并发症及相似或更好的临床结果。单位体积内过大移植容量,可能是导致手术效果不佳的重要原因。过长手术时间、过多细胞移植穿刺注射点,可能对手术效果产生负面影响。手术规范初步提示:① 细胞的浓度:适中(1~3万/μL);② 细胞的总量:适中(>1,000,000/50~100μL);③ 切口选择:微创、锁孔(key hole);④ 移植的部位:正常异常交界区(可植入至足够容积的损伤区);⑤ 移植点的数目:4~8个;⑥ 移植器械选择与使用方式:适合灭菌,器械+徒手配合;⑦ 术中粘连及囊肿等处理:建议实施;⑧ 术后管理:早期足量目标导向科学康复。

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哌齐在运动性失语的后期恢复中的作用值得在功能磁共振下进一步探讨。

本实验首次利用任务态的功能磁共振结合临床观察，研究多奈哌齐对急性缺血性脑卒中后运动性失语患者的疗效，探讨其促进脑言语功能区损伤后的恢复机制，为多奈哌齐治疗运动性失语提供理论依据。

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