电针夹脊穴和督脉穴对急性脊髓损伤家兔后肢神经功能的影响及其作用机制

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摘　要　目的:观察电针夹脊穴和督脉穴对急性脊髓损伤家兔后肢神经功能的影响,并探讨其作用机制。**方法:**采用随机数字表将 60 只新西兰家兔随机分为假手术组、模型组、电针组,每组 20 只。假手术组咬除 T_{13} ~ L_1 棘突及全部椎板,暴露 0.5 cm 宽硬膜,不损伤脊髓;模型组和电针组采用改良 Allens 法建立急性脊髓损伤家兔模型,并行脊髓 MRI 扫描鉴定模型。模型组和假手术组不给予治疗,电针组给予电针刺激受损脊髓节段上下两对夹脊穴及上下督脉穴(筋缩、命门)治疗,每次 30 min,每日 1 次,共 7 次。电针干预开始后 1 d、3 d、7 d 采用改良 Tarlov 评分法评价家兔后肢神经功能。干预结束后每组取 10 只家兔,以 4% 多聚甲醛行心脏灌注后剖取损伤脊髓约 1 cm,制成切片后经 HE 染色于显微镜下观察脊髓组织形态,并采用免疫组织化学法观察脊髓细胞增殖情况和巢蛋白(Nestin)表达水平。将每组剩余的 10 只家兔麻醉,分离坐骨神经并注入 5 μL 的 30% 辣根过氧化物酶(horseradish peroxidase,HRP),24 h 后取脊髓横断面背侧靠近损伤处脊髓,采用 HRP 逆行示踪法标记红核神经元细胞数。**结果:**①模型鉴定结果。MRI 片上假手术组脊髓横断面信号正常,可见清晰的脑脊液通过。模型组和电针组脊髓横断面信号增强,脑脊液受压;受伤节段的脊髓在矢状位 MRI 片 T2WI 上呈明显的高信号,T1WI 上呈低信号;提示脊髓断端周围充血、水肿,证明造模成功。②后肢神经功能评分。电针干预后不同时间点 Tarlov 评分比较,差异有统计学意义,即存在时间效应($F=6.920$,$P=0.001$);除假手术组外,干预后模型组和电针组 Tarlov 评分均逐渐升高。3 组 Tarlov 评分总体上比较,组间差异有统计学意义,即存在分组效应($F=426.78$,$P=0.000$)。干预开始后 1 d,电针组 Tarlov 评分高于模型组[(0.30 ± 0.47)分,(0.20 ± 0.41)分,$q=7.010$,$P=0.486$],低于假手术组[(0.30 ± 0.47)分,(4.70 ± 0.47)分,$q=30.843$,$P=0.000$];模型组低于假手术组($q=31.544$,$P=0.000$)。干预开始后 3 d,电针组 Tarlov 评分高于模型组[(1.55 ± 0.60)分,(1.05 ± 0.53)分,$q=2.877$,$P=0.006$],低于假手术组[(1.55 ± 0.60)分,(4.80 ± 0.41)分,$q=20.775$,$P=0.000$];模型组低于假手术组($q=23.651$,$P=0.000$)。干预开始后 7 d,电针组 Tarlov 评分高于模型组[(2.65 ± 0.59)分,(1.40 ± 0.50)分,$q=8.004$,$P=0.000$],低于假手术组[(2.65 ± 0.59)分,(4.85 ± 0.41)分,$q=14.081$,$P=0.000$];模型组低于假手术组($q=22.093$,$P=0.000$)。时间因素和分组因素之间存在交互效应($F=70.786$,$P=0.000$)。③脊髓组织形态。假手术组脊髓形态结构完整,神经元结构正常、分布均匀,细胞膜、细胞核及组织间隙均正常,尼氏体显示清晰;模型组脊髓组织正常结构丧失,可见组织充血、水肿,神经元细胞数量减少,部分尼氏体消失,灰质中部分区域甚至有较大的空腔;电针组与模型组相比,脊髓形态结构基本完整,神经元细胞结构基本正常,空泡变性减少,组织充血、水肿消失。④脊髓细胞增殖情况。电针干预开始后 7 d,200 倍显微镜下观察,每个视野下 3 组家兔脊髓增殖细胞数比较,组间差异有统计学意义[(43.67 ± 8.19)个,(18.77 ± 3.55)个,(6.30 ± 2.32)个,$F=382.413$,$P=0.000$];电针组脊髓增殖细胞多于模型组($q=20.889$,$P=0.000$)和假手术组($q=31.348$,$P=0.000$),模型组多于假手术组($q=10.459$,$P=0.000$)。⑤脊髓细胞 Nestin 表达水平。干预开始后 7 d,200 倍显微镜下观察,每个视野下 3 组家兔脊髓细胞 Nestin 阳性表达细胞数比较,组间差异有统计学意义[(23.37 ± 5.10)个,(10.77 ± 3.26)个,(4.43 ± 1.33)个,$F=218.084$,$P=0.000$];电针组 Nestin 阳性表达细胞数多于模型组($q=15.770$,$P=0.000$)和假手术组($q=23.696$,$P=0.000$),模型组多于假手术组($q=7.926$,$P=0.000$)。⑥红核神经元细胞数。干预开始后 7 d,200 倍显微镜下观察,每个视野下 3 组红核神经元细胞数比较,组间差异有统计学意义(513.40 ± 46.79,417.90 ± 43.03,578.40 ± 43.91,$F=32.760$,$P=0.000$);电针组红核神经元细胞数多于模型组($q=6.771$,$P=0.000$)、少于假手术组($q=4.608$,$P=0.000$),模型组少于假手术组($q=11.379$,$P=0.000$)。**结论:**电针刺激夹脊穴和督脉穴能明显改善急性脊髓损伤家兔的后肢神经功能,其作用机制可能是通过促进神经干细胞增殖分化和抑制红核神经元细胞凋亡,从而使损伤节段神经组织修复及神经通路再通。

关键词　脊髓损伤;电针;穴,夹脊;穴位,督脉;神经干细胞;神经传导;兔;动物实验

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Effects of electroacupuncture at Point JIAJI(EX – B2) and Point governor vessel on neural function of hindlimbs in rabbits with acute spinal cord injury and the mechanism of action

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ABSTRACT　Objective:To observe the effects of electroacupuncture at Point JIAJI(EX – B2) and Point governor vessel on neural function of hindlimbs in rabbits with acute spinal cord injury and to explore its mechanism of action. **Methods**:Sixty New Zealand rabbits were randomly divided into sham – operated group,model group and electroacupuncture group,20 cases in each group. The random digits table was used for grouping. The spinous processes and ertebral plates of T_{13} – L_1 of rabbits in sham – operated group were removed to expose 0.5 cm of endorachis in width without spinal cord injuries,while the models of acute spinal cord injury were built in rabbits in model group and electroacupuncture group by using improved Allens method and the models were identified by spinal cord MRI scanning. The rabbits in model group and sham – operated group were untreated,while the rabbits in electroacupuncture group were treated with electroacupuncture at two pairs of Point JIAJI(EX – B2) and upper and lower Point governor vessel(Point JINSUO and Point MINGMEN) at the injured segments of spinal cord,30 minutes at a time,once a day for consecutive 7 times. At 1,3 and 7 days after the beginning of electroacupuncture intervention,the neural function of hindlimbs of rabbits were evaluated by using improved Tarlov scoring method. After the end of intervention,10 rabbits were randomly selected from each group and were treated by perfusing their hearts with 4% paraformaldehyde. Then approximate 1 cm injured spinal cord were fetched out and sectioned for HE staining. The spinal cord tissue morphology were observed under the microscope and the proliferations of spinal cord cells and Nestin expression levels were detected by using immunohistochemical method. The sciatic nerves of the remaining 10 rabbits in each group were separated under anesthesia and 30% horseradish peroxidase(HRP) were injected into the sciatic nerves in dosage of 5 μL. Twenty – four hours later,the dorsal spinal cord tissues near the injuried spinal cord were fetched out and the numbers of red nucleus neuronal cells were marked by using HRP retrograde tracing technique. **Results**:The MRI signals of spinal cord in the transverse plane were normal and clear cerebrospinal fluids were found in sham – operated group;while enhanced MRI signals of spinal cord in transverse plane and compressed cerebrospinal fluids were found in model group and electroacupuncture group,and the injured segments of spinal cord presented with obvious high signal on T2 – weighted MRI and low signal on T_1 – weighted MRI in the sagittal plane,which suggested that there were engorgement and edema arround the broken ends of spinal cord and the models were successfully built. There was statistical difference in Tarlov scores between different time points after electroacupuncture intervention,in other words,there was time effect($F=6.920$,$P=0.001$). The Tarlov scores increased gradually in model group and electroacupuncture group after intervention. There was statistical difference in Tarlov scores between the 3 groups in general,in other words,there was group effect($F=426.78$,$P=0.000$). At 1 day after the beginning of intervention,the Tarlov scores were higher in electroacupuncture group compared to model group(0.30 +/– 0.47 vs 0.20 +/– 0.41 points,$q=7.010$,$P=0.486$) and were lower in electroacupuncture group compared to sham – operated group(0.30 +/– 0.47 vs 4.70 +/– 0.47 points,$q=30.843$,$P=0.000$) and were lower in model group compared to sham – operated group($q=31.544$,$P=0.000$). At 3 days after the beginning of intervention,the Tarlov scores were higher in electroacupuncture group compared to model group(1.55 +/– 0.60 vs 1.05 +/– 0.53 points,$q=2.877$,$P=0.006$) and were lower in electroacupuncture group compared to sham – operated group(1.55 +/– 0.60 vs 4.80 +/– 0.41 points,$q=20.775$,$P=0.000$),and the Tarlov scores were lower in model group compared to sham – operated group($q=23.651$,$P=0.000$). At 7 days after the beginning of intervention,the Tarlov scores were higher in electroacupuncture group compared to model group(2.65 +/– 0.59 vs 1.40 +/– 0.50 points,$q=8.004$,$P=0.000$) and were lower in electroacupuncture group compared to sham – operated group(2.65 +/– 0.59 vs 4.85 +/– 0.41 points,$q=14.081$,$P=0.000$),and the Tarlov scores were lower in model group compared to sham – operated group($q=22.093$,$P=0.000$). There was interaction between time factor and group factor($F=70.786$,$P=0.000$). In sham – operated group,the shape and structure of spinal cord were complete and the neurons were uniformly distributed in normal structure;the cellular membrane,cellular nucleus and tissue spaces were normal and Nissl bodies were clear. In model group,the normal structure of spinal cord tissue disappeared,and hyperaemia and edema in the tissues and decrease of number of neurons were found;some Nissl body disappeared and there were big cavity in some areas of grey matter. In electroacupuncture group,the shape and structure of spinal cord were basically complete and the structure of neuron was ba-

sically normal;the vacuolar degeneration decreased and hyperaemia and edema in the tissues disappeared. At 7 days after the beginning of electroacupuncture intervention,there was statistical difference in the number of proliferative spinal cord cells under the optical microscope (×200) between the 3 groups(43.67 +/- 8.19,18.77 +/- 3.55,6.30 +/- 2.32,F = 382.413,P = 0.000);the number of proliferative spinal cord cells was more in electroacupuncture group compared to model group(q = 20.889,P = 0.000) and sham - operated group(q = 31.348,P = 0.000),and the number of proliferative spinal cord cells was more in model group compared to sham - operated group(q = 10.459,P = 0.000). At 7 days after the beginning of intervention,there was statistical difference in the number of Nestin positive expression cells of spinal cord under the optical microscope(×200) between the 3 groups(23.37 +/- 5.10,10.77 +/- 3.26,4.43 +/- 1.33,F = 218.084,P = 0.000);the number of Nestin positive expression cells was more in electroacupuncture group compared to model group(q = 15.770,P = 0.000) and sham - operated group(q = 23.696,P = 0.000),and the number of Nestin positive expression cells was more in model group compared to sham - operated group(q = 7.926,P = 0.000). At 7 days after the beginning of intervention,there was statistical difference in the number of red nucleus neuronal cells under the optical microscope(×200) between the 3 groups(513.40 +/- 46.79,417.90 +/- 43.03,578.40 +/- 43.91,F = 32.760,P = 0.000);the number of red nucleus neuronal cells was more in electroacupuncture group compared to model group(q = 6.771,P = 0.000) and was less compared to sham - operated group(q = 4.608,P = 0.000),and the number of red nucleus neuronal cells was less in model group compared to sham - operated group(q = 11.379,P = 0.000). **Conclusion**: Electroacupuncture at Point JIAJI(EX - B2) and Point governor vessel can obviously improve the neural function of hindlimbs in rabbits with acute spinal cord injury. It can promote the differentiation and proliferation of neural stem cells and inhibit the apoptosis of red nucleus neuronal cells,which may be the mechanisms of action for repairing the nervous tissues at the injuried segments and recirculating the neural pathway.

Key words　spinal cord injuries;electroacupuncture;point EX - B2(JIAJI);points,governor vessel;neural stem cells;neural conduction;rabbits;animal experimentation

脊髓损伤是因各种原因引起脊髓结构、功能的损伤,造成损伤平面以下脊髓功能的障碍[1-2]。据统计,每年全球脊髓损伤的发病率为每百万人 20 ~ 60 例,而脊髓损伤后 25 年病死率约为 49%[3-4]。本研究观察电针刺激急性脊髓损伤家兔的夹脊穴和督脉穴后,其后肢神经功能、脊髓形态、脊髓细胞增殖和巢蛋白(Nestin)表达及红核神经元细胞逆行性损伤情况,以探讨电针对家兔脊髓损伤后后肢神经功能的影响及其作用机制,为针刺治疗急性脊髓损伤提供科学的实验依据。

1　材料与仪器

1.1　实验动物　3 月龄健康新西兰家兔 60 只,雌雄各半,体质量 2.0 ~ 3.0 kg,由浙江中医药大学动物实验中心提供,实验动物许可证号:SCXK(沪)2013 - 2016。实验方案通过医学动物实验伦理委员会批准。

1.2　实验试剂　辣根过氧化物酶(horseradish peroxidase,HRP;sigma 公司),多聚甲醛液、二甲苯、无水乙醇、30% 过氧化氢(上海国药集团),牛血清白蛋白(北京索莱宝生物科技有限公司),3,3 - 二氨基苯联胺(diaminobenzidine,DAB)浓缩型试剂盒、中性树脂、二抗(上海长岛生物技术有限公司),溴脱氧尿苷(bromodeoxyuridine,BrdU)、Nestin 试剂盒(Abcam 公司)。

1.3　实验仪器　一次性华佗牌针灸针、电针仪(苏州医疗用品厂),正置显微镜(Olympus 公司),冷冻切片机(徕克公司),IMS 图象分析系统(基尔顿生物科技有限公司)。

2　方　法

2.1　分组与造模　适应性饲养 1 周后采用随机数字表将 60 只家兔随机分为假手术组、模型组、电针组,每组 20 只。模型组和电针组采用改良 Allens 法[5-6]建立急性脊髓损伤家兔模型:术区常规剪毛、消毒,耳缘静脉注射 3% 戊巴比妥进行麻醉;家兔取俯卧位,以 T_{13} 为中心取后背正中切口,暴露上下各 1 个椎体长度,咬除 T_{13} ~ L_1 棘突及全部椎板,暴露 0.5 cm 宽的硬膜;用质量为 10 g 的克氏针沿带有刻度的导管从 80 mm 高处垂直自由下落,打击在直径 4 mm、厚2 mm 的由薄塑料材料制成的半圆片上,致伤后迅速移开打击物,造成脊髓后角中度损伤(打击后兔子尾巴呈痉挛性摆动,双下肢及躯体回缩样扑动,麻醉清醒后双下肢呈迟缓性瘫痪),最后逐层缝合切口。干预后每天采用 Crede 手法[7]按摩家兔腹部,辅助排便、排尿 2 ~ 4次,直至反射性膀胱排空建立。假手术组仅咬除 T_{13} ~ L_1 棘突及全部椎板,暴露 0.5 cm 宽的硬膜,不

损伤脊髓。各组家兔手术后每天肌肉注射 80 万单位青霉素预防切口感染,连续注射 2 d。

2.2　模型鉴定　造模后采用西门子 1.5 T 超导 MRI 成像仪行脊髓矢状面、横断面 MRI 扫描鉴定模型。环形表面线圈,扫描序列包括:T1WI 采用 SE 序列,T2WI 及质子密度加权采用 TSE 序列。T1WI 扫描参数为 TR = 615 ms、TE = 14 ms;T2WI 扫描参数为TR = 3750 ms、TE = 107 ms、层厚 3 mm、层距 0.3 mm、视野 200 mm × 200 mm。

2.3　电针干预　模型组和假手术组不给予治疗。电针组建立急性脊髓损伤模型后给予电针治疗:用直径 0.25 mm 的针灸针针刺受损脊髓节段上下两对夹脊穴及上下督脉穴(筋缩、命门),深度为 10 mm;然后将针灸针连接至电针仪上,采用连续脉冲,频率 2 Hz,输出电流 0.6 ~ 1 mA,刺激 30 min,每日 1 次,共 7 次,电流以局部出现轻度跳动且家兔能耐受为度。

2.4　改良 Tarlov 评分法评价后肢神经功能　电针干预开始后 1 d、3 d、7 d 采用改良 Tarlov 评分法[8]对家兔后肢神经功能进行评定,即分别对兔双侧后肢进行功能评估,每项指标共测定 3 次,取平均值。0 级(0 分):无自主性运动;1 级(1 分):仅限于髋、膝关节的非反射性运动;2 级(2 分):肢体髋、膝、踝 3 个主要关节的运动;3 级(3 分):能主动支持体重和不协调步态,或偶尔出现协调步态;4 级(4 分):前肢和后肢协调的步态,行走时有趾间关节的运动;5 级(5 分):正常步态。

2.5　脊髓组织取材和形态观察　电针干预开始后 7 d,每组取 10 只家兔进行取材。于取材前 3 d 连续腹腔注射浓度为 10 mg · mL^{-1}的 BrdU,每次剂量为 50 mg · kg^{-1},每天 2 次,每次间隔 8 h。最后 1 次给药 2 h 后予以麻醉;采用胸部正中纵形切口逐层切开,切断肋骨,显露心脏;切开左心室及右心房,从左心室沿主动脉的方向插管,快速灌入生理盐水直至回右心房的灌注液清亮后,灌入 4% 的多聚甲醛液,流量为 50 mL · min^{-1}维持 3 ~ 4 min,然后流量为 10 mL · min^{-1}维持约 20 min;成功行心脏灌注后,截取脊髓损伤节段的椎体,在冰上剖取损伤脊髓约 1 cm,用生理盐水冲洗,置于 4% 多聚甲醛中固定,4 ℃过夜,梯度酒精脱水,石蜡包埋后,制成 6 μm 切片。石蜡切片脱蜡至水,苏木素染色 5 min,伊红染色液复染 2 min,用中性树胶封片后,于 100 倍正置显微镜下观察脊髓组织形态学变化。

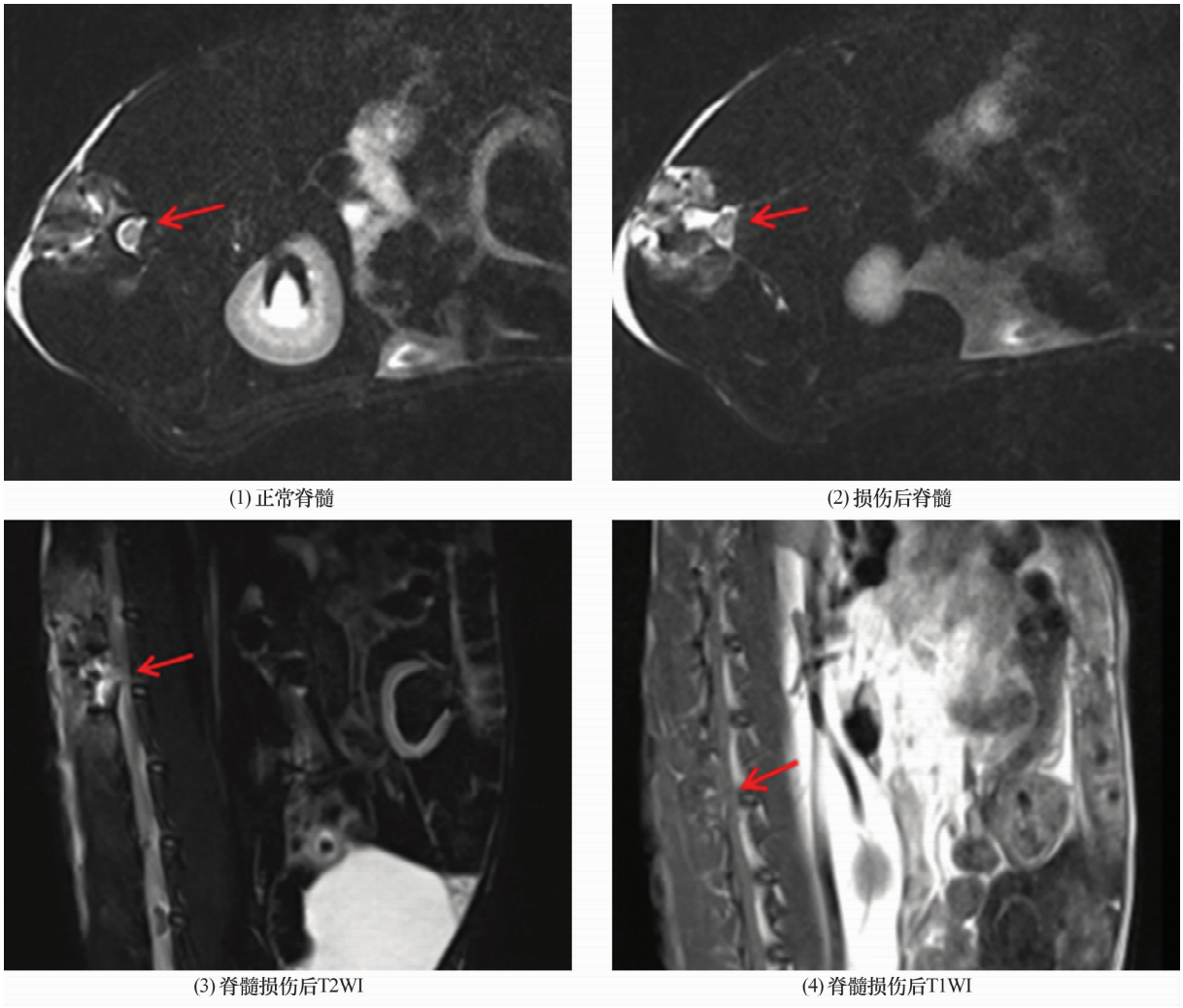
2.6　免疫组织化学法观察脊髓细胞增殖和 Nestin 表达水平　电针干预开始后 7 d 各组目标节段脊髓组织冰冻切片 6 μm,4% 多聚甲醛溶液固定 30 min,BrdU染色需进行 DNA 变性,Nestin 无需变性,3% 过氧化氢阻断。加一抗,4 ℃孵育过夜,磷酸盐缓冲液漂洗,加 HRP 标记广谱二抗 37 ℃孵育 20 min。DAB 染色,苏木精复染 3 min,二甲苯透明,用中性树胶封片后,于 200 倍显微镜下观察拍照。每组每只兔子各选取 1 个样品进行观察,每个样品随机选取 3 个视野(×200)进行阳性细胞计数,并取平均值作为该样品的平均阳性细胞数供统计使用。

2.7　HRP 逆行示踪法标记红核神经元细胞数　电针干预开始后 7 d 每组取剩余的 10 只家兔麻醉,剪掉后腿部的毛,常规消毒皮肤,逐层切开皮肤,仔细分离肌肉寻找坐骨神经,并用微量注射器将约 5 μL 的 30% HRP 缓慢注入坐骨神经,留针 10 min。24 h 后取脊髓横断面背侧靠近损伤处脊髓,4% 多聚甲醛固定 8 ~ 12 h后冷冻切片,行 HRP - DAB - 硫酸镍胺增强法显色后,于 200 倍显微镜下观察。采用 IMS 图象分析系统采集图片,每个样品随机选取 3 个视野(×200),拍照并保存。使用 Image - Pro Plus 6.0 软件对图片进行图像分析,测出图像阳性表达累积光密度(integrated optical density,IOD)、目标组织区域面积(Area),并计算出各图片的平均光密度(ODmean),计算公式如下:ODmean = IOD/Area。再计算出每个样品 3 个视野的平均值作为该样品的平均光密度供统计使用。

2.8　数据统计学分析　采用 SPSS 22.0 统计软件对所得数据进行统计学处理。电针干预后不同时间点 3 组家兔后肢神经功能 Tarlov 评分的比较采用重复测量资料的方差分析,3 组家兔脊髓增殖细胞数、Nestin 阳性表达细胞数、红核神经元细胞数的组间比较采用单因素方差分析,组间两两比较采用 q 检验,检验水准 $\alpha = 0.05$。

3　结　果

3.1　模型鉴定结果　假手术组脊髓横断面的信号正常,可见清晰的脑脊液通过[图 1(1)]。模型组和电针组脊髓横断面信号增强,脑脊液受压[图 1(2)];受伤节段的脊髓在矢状位 MRI 片 T2WI 上呈明显的高信号[图 1(3)],T1WI 上呈低信号[图 1(4)];提示脊髓断端周围充血、水肿,证明造模成功。



(1) 正常脊髓　(2) 损伤后脊髓

(3) 脊髓损伤后T2WI　(4) 脊髓损伤后T1WI

图 1　家兔脊髓急性损伤前后 MRI

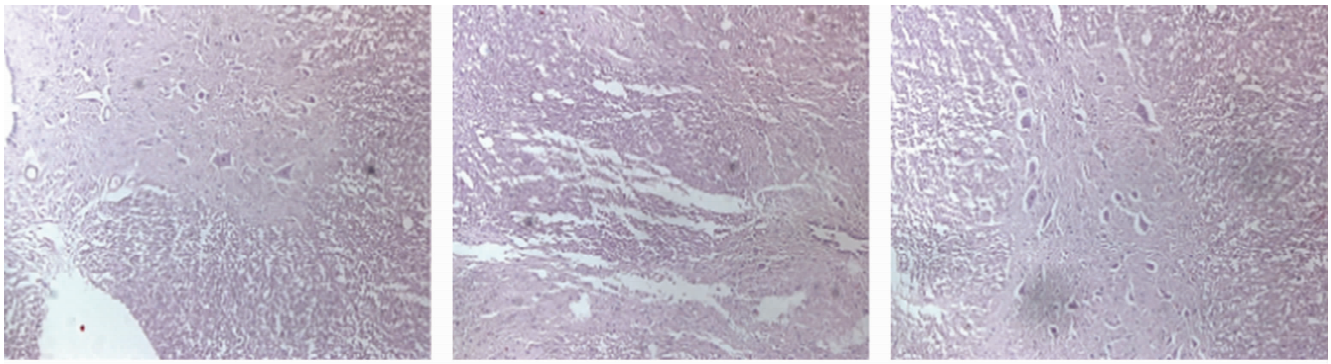
3.2　后肢神经功能评分　电针干预后不同时间点 Tarlov 评分比较，差异有统计学意义，即存在时间效应；除假手术组外，干预后模型组和电针组 Tarlov 评分均逐渐升高。3 组 Tarlov 评分总体上比较，组间差异有统计学意义，即存在分组效应。干预开始后 1 d，电针组 Tarlov 评分高于模型组（$q=7.010$，$P=0.486$），低于假手术组（$q=30.843$，$P=0.000$）；模型组低于假手术组（$q=31.544$，$P=0.000$）。干预开始后 3 d，电针组 Tarlov 评分高于模型组（$q=2.877$，$P=0.006$），低于假手术组（$q=20.775$，$P=0.000$）；模型组低于假手术组（$q=23.651$，$P=0.000$）。干预开始后 7 d，电针组 Tarlov 评分高于模型组（$q=8.004$，$P=0.000$），低于假手术组（$q=14.081$，$P=0.000$）；模型组低于假手术组（$q=22.093$，$P=0.000$）。时间因素和分组因素之间存在交互效应。见表 1。

表 1　3 组家兔后肢神经功能 Tarlov 评分比较

组别	样本量（只）	神经功能 Tarlov 评分（$\bar{x}\pm s$，分）				F 值	P 值
		电针干预开始后 1 d	电针干预开始后 3 d	电针干预开始后 7 d	合计		
假手术组	20	4.70±0.47	4.80±0.41	4.85±0.41	4.78±0.42	0.668	0.517
模型组	20	0.20±0.41	1.05±0.53	1.40±0.50	0.88±0.67	28.268	0.000
电针组	20	0.30±0.47	1.55±0.60	2.65±0.59	1.50±1.11	95.386	0.000
合计	60	1.73±2.16	2.47±1.74	2.97±1.52	2.39±1.89	6.920[1)]	0.001[1)]
F 值		648.948	360.782	250.212	426.78[1)]	$F=70.786$[2)]，	
P 值		0.000	0.000	0.000	0.000[1)]	$P=0.000$[2)]	

1）主效应的 F 值和 P 值；2）交互效应的 F 值和 P 值

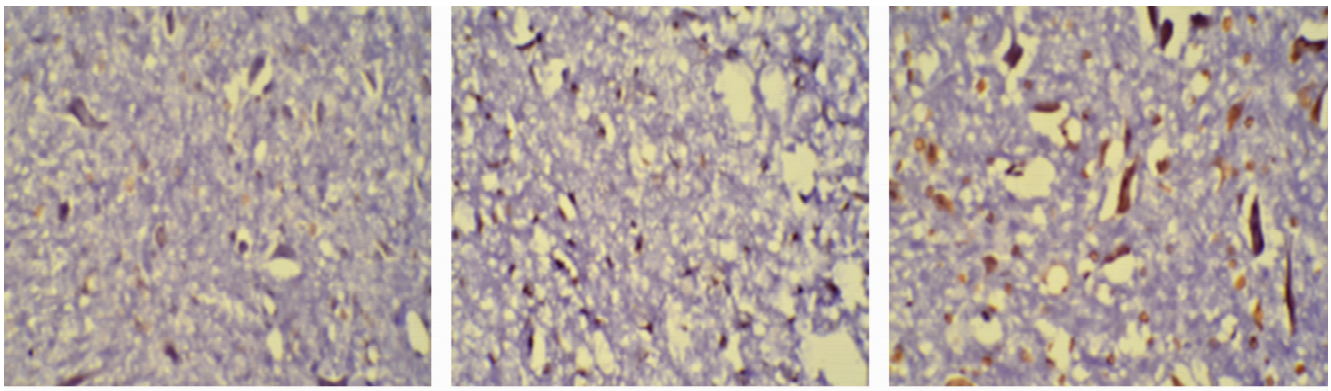
3.3　脊髓组织形态　假手术组脊髓形态结构完整，神经元结构正常，分布均匀，细胞膜、细胞核及组织间隙均正常，尼氏体显示清晰[图 2(1)]；模型组脊髓组织正常结构丧失，可见组织充血、水肿，神经元细胞数量减少，部分尼氏体消失，灰质中部分区域甚至有较大的空腔[图 2(2)]；电针组与模型组相比，脊髓形态结构基本完整，神经元细胞结构基本正常，空泡变性减少，组织充血、水肿消失[图 2(3)]。



(1) 假手术组　(2) 模型组　(3) 电针组

图 2　显微镜下 3 组家兔脊髓组织形态(HE 染色　×100)

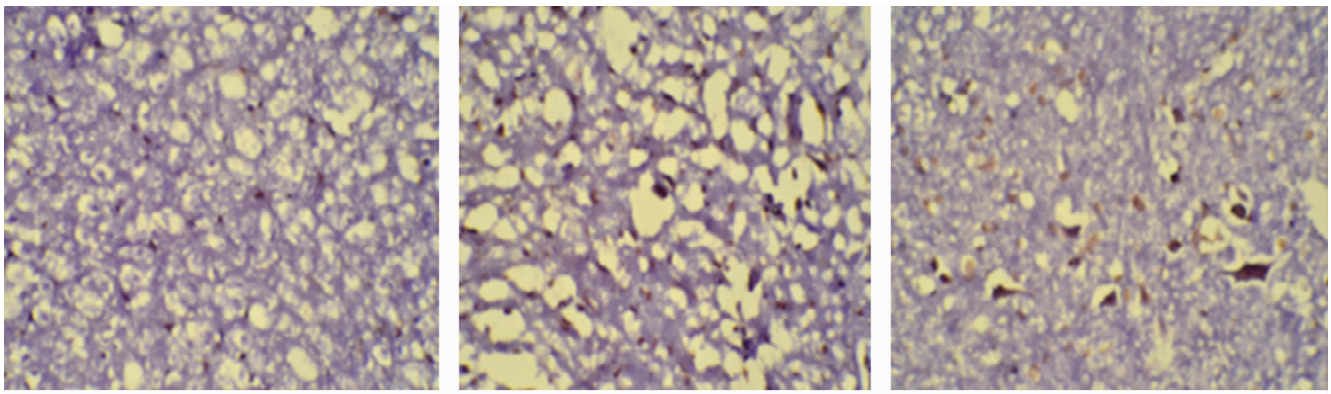
3.4　脊髓细胞增殖情况　干预开始后 7 d，3 组家兔脊髓增殖细胞数比较，组间差异有统计学意义；电针组脊髓增殖细胞多于模型组($q = 20.889$，$P = 0.000$)和假手术组($q = 31.348$，$P = 0.000$)，模型组多于假手术组($q = 10.459$，$P = 0.000$)。见图 3、表 2。



(1) 假手术组　(2) 模型组　(3) 电针组

图 3　显微镜下 3 组家兔脊髓增殖细胞(免疫组化染色　×200)

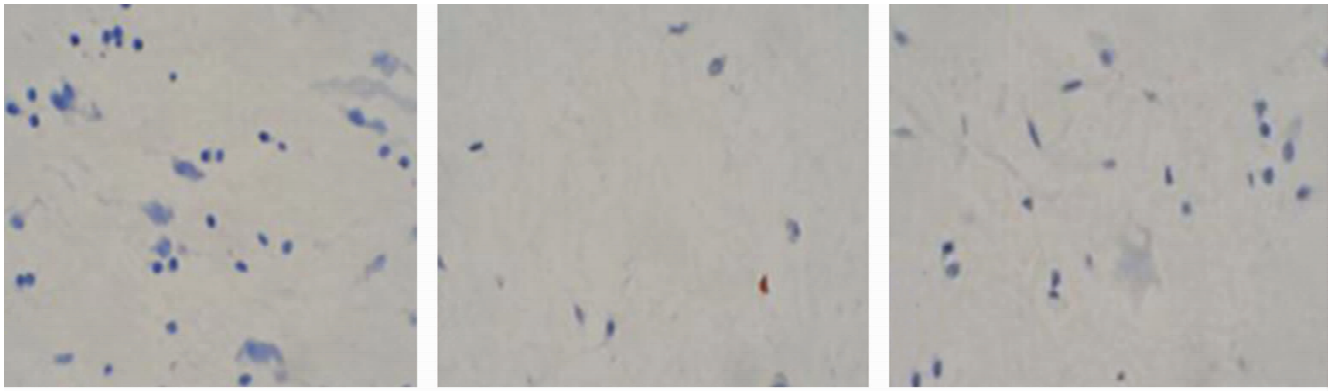
3.5　脊髓细胞 Nestin 表达水平　电针干预开始后 7 d，3 组 Nestin 阳性表达细胞数比较，组间差异有统计学意义；电针组 Nestin 阳性表达细胞数多于模型组($q = 15.770$，$P = 0.000$)和假手术组($q = 23.696$，$P = 0.000$)，模型组多于假手术组($q = 7.926$，$P = 0.000$)。见图 4、表 2。



(1) 假手术组　(2) 模型组　(3) 电针组

图 4　显微镜下 3 组家兔脊髓细胞 Nestin 蛋白表达结果(免疫组化染色　×200)

3.6　红核神经元细胞数　电针干预开始后 7 d,3 组红核神经元细胞数比较,组间差异有统计学意义;电针组红核神经元细胞数多于模型组(q = 6.771,P = 0.000)、少于假手术组(q = 4.608,P = 0.000),模型组少于假手术组(q = 11.379,P = 0.000)。见图 5、表 2。



(1) 假手术组　(2) 模型组　(3) 电针组

图 5　显微镜下 3 组家兔红核神经元细胞(HRP 逆行示踪染色　×200)

表 2　3 组家兔脊髓增殖细胞、Nestin 阳性表达细胞及红核神经元细胞数量比较　$\bar{x} \pm s$

组别	样本量（只）	200 倍显微镜每个视野下的细胞数		
		脊髓增殖细胞（个）	脊髓 Nestin 阳性表达细胞（个）	红核神经元细胞
假手术组	10	6.30 ± 2.32	4.43 ± 1.33	578.40 ± 43.91
模型组	10	18.77 ± 3.55	10.77 ± 3.26	417.90 ± 43.03
电针组	10	43.67 ± 8.19	23.37 ± 5.10	513.40 ± 46.79
F 值		382.413	218.084	32.760
P 值		0.000	0.000	0.000

红核神经元细胞数以平均光密度表示

4　讨　论

脊髓损伤具有死亡率高、致残率高、康复难、病程长、治疗费用高等特点,因此其预防、治疗和康复日益引起中外学者们的高度关注。脊髓损伤属中医学“痿证”范畴,其病因病机主要是督脉受损。电针具有不良反应少等优点,易于患者接受。采用电针刺激督脉经穴和夹脊穴治疗脊髓损伤,符合“治病必求其本”的原则;既可直达病所疏通督脉使阳气通达,又可获得调理气血、行气活血生髓的功效[9];还可以改善因脊髓损伤所导致的尿潴留,神经、运动功能障碍,神经性、病理性疼痛,瘢痕形成等并发症和后遗症[10-14]。

BrdU 标记和检测的准确性高、方法简便,是反映细胞增殖的理想指标,也是体内标记神经干细胞增殖、迁移的重要手段[15]。Nestin 属于第Ⅳ类中间丝蛋白,具有维持神经前体细胞正常形态的作用,是神经系统结构和功能发育的关键因素[16]。脊髓损伤后,红核脊髓束可以发生延迟性的变性坏死,红核神经元继而凋亡,这种病理变化将严重影响脊髓神经功能的恢复。HRP 逆行示踪技术可以对脊髓损伤后的神经修复状况进行可靠的形态学评估[17-18]。

本研究采用节段叠加配穴及中轴贯通配穴法[19-20],选取损伤节段上下的两对夹脊穴及 2 个督脉穴,进行连续波电针刺激。结果显示,电针组经过 7 d 的干预,后肢神经功能得到了一定的改善,Tarlov 评分高于模型组;脊髓形态结构趋向完整,神经元细胞结构基本正常,组织充血、水肿消失;脊髓增殖细胞明显多于模型组,Nestin 阳性表达细胞明显多于模型组,神经组织得到了一定的修复;HRP 逆行标记的红核神经元细胞数也明显高于模型组。这间接提示了内源性神经干细胞的增殖、红核损伤程度与神经功能的恢复有着紧密的联系。其作用机制可能是通过电针刺激损伤脊髓上下节段的神经根,将电流贯通损伤脊髓区域,从而激发内源性神经干细胞潜在的增殖、分化能力,诱导其向神经元方向分化以替代受损神经元的功能,促进受损的神经组织进行功能自我修复;又抑制了红核神经元细胞的凋亡,促进神经通路修复和再生。

本研究结果显示,电针刺激夹脊穴和督脉穴能明

显改善急性脊髓损伤家兔的后肢神经功能,其作用机制可能是通过促进神经干细胞增殖分化和抑制红核神经元细胞凋亡,从而使损伤节段神经组织修复及神经通路再通。

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为了保证学术论文的真实性和原创性,杜绝学术论文抄袭、剽窃、伪造、篡改、不当署名、一稿多投等现象的发生,本着对本刊论文作者和读者负责的态度,《中医正骨》编辑部于2010年4月开始启用《科技期刊学术不端文献检测系统(AMLC)》。该系统以《中国学术文献网络出版总库》为全文比对数据库,本刊编辑使用该系统对所有来稿的文字复制情况进行检测,检测结果包括与已发表论文比对后的文字复制比率,所涉及论文的题目、作者、发表期刊和发表时间。按规定文字复制比超过20%的来稿即视为存在学术不端行为的可能,经人工比对后才能进入下一个审稿程序,特此提醒广大作者,注意所投稿件的原创性与真实性。特此通知。