·论著·

富血小板血浆联合骨髓间充质干细胞对大鼠脊髓损伤后 NGF、BDNF 的影响

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摘要: **目的** 观察富血小板血浆(PRP)与骨髓间充质干细胞(BMSCs)联合应用对大鼠脊髓损伤后神经营养因子的神经生长因子(NGF)、脑源性神经营养因子(BDNF)的影响。**方法** SD 大鼠 40 只,建立大鼠脊髓损伤模型,术后 PRP 组在损伤段脊髓注入 RPR,BMSCs 组注入 BMSCs,PRP + BMSCs 联合组注入 BMSCs + PRP 复合物,对照组不注入任何物质。术前 3d 和术后 8 周对大鼠后肢的恢复能力进行 BBB 运动功能评分。造模后第 8 周,处死动物,HE 染色进行病理学检查。蛋白质印迹法(Western blot)检测脊髓中 NGF、BDNF 蛋白水平的变化。**结果** 与对照组相比,各治疗组大鼠后肢功能恢复具有明显提高,PRP + BMSCs 组的大鼠后肢功能恢复最好,差异有显著性意义($P < 0.05$)。与对照组相比,各治疗组脊髓的 NGF、BDFN 的蛋白表达水平均有所提高,差异有显著性意义($P < 0.05$)。**结论** PRP、BMSC 能恢复脊髓损伤大鼠后肢功能恢复,提高脊髓中 NGF、BDNF 蛋白水平。

关键词: 富血小板血浆;骨髓间充质干细胞;脊髓损伤;NGF;BDNF

Effect of platelet rich plasma combined with bone marrow mesenchymal stem cells on the expression of nerve growth factor and brain-derived neurotrophic factor in rats with spinal cord injuries

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Abstract: **Objective** To observe the effect of platelet rich plasma (PRP) combined with bone marrow mesenchymal stem cells (BMSCs) on the expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in rats with spinal cord injuries. **Methods** The rat model with spinal cord injury was established using 40 SD rats. After the operation, rats in PRP group were injected with PRP into the spinal cord at the injured segments. Rats in BMSCs group were injected with BMSCs, and rats in PRP + BMSCs combination group were injected with the combination of BMSCs and PRP, while rats in the control group were treated without any injection. At the 3rd day and the 8th week after the operation, the BBB motor function of the hind limbs of rats was evaluated. At the 8th week after model establishment, the rats were executed and the HE staining was performed for pathological examination. The changes of the expression of NGF and BDNF protein were detected using Western blotting. **Results** Compared with that in the control group, the function of the hind limbs of rats in each treatment group improved significantly, and the biggest improvement was observed in PRP + BMSCs combination group ($P < 0.05$). The expression of NGF and BDNF protein in spinal cord in each treatment group improved significantly comparing with that in the control group ($P < 0.05$). **Conclusion** PRP and BMSCs can restore the function of the hind limbs of rats with spinal cord injuries and improve the expression of NGF and BDNF protein in spinal cord.

Key words: PRP; BMSCs; Spinal cord injury; NGF; BDNF

脊髓损伤(spinal cord injury, SCI)是指由于外界直接或间接因素导致脊髓损伤,在损害的相应节段出现各种运动、感觉和括约肌功能障碍,肌张力异

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常及病理反射等的相应改变。脊柱损伤、脊椎退变及脊髓缺血等均可造成脊髓损伤。脊髓损伤具有高发生率、高致残率、高耗费、低病死率的特点。近年来研究发现,脊髓损伤的发病情况在逐年增加,对人们健康的生活质量造成了严重的威胁。目前对脊髓损伤的治疗方法主要有药物治疗和外科治疗等,这些方法虽在不同程度上对脊髓损伤起到了一定的治疗作用,但其治疗效果仍难以获得较满意的疗效[1]。本实验研究 PRP 联合 BMSCs 对大鼠脊髓损伤后 NGF、BDNF 蛋白水平的变化,为更深入研究提供科学的基础数据。

1 材料与方法

1.1 实验动物及分组

SD 大鼠,雄性,180 ~ 220 g,SPF 级,由广西医科大学实验动物中心提供,试验动物生产许可证 SCXK(桂)2009-0002,试验动物使用许可证 SCXK(桂)2009-0005。将 40 只 SD 大鼠随机分为 4 组,每组 10 只:对照组、PRP 组、BMSCs 组、PRP + BMSCs 组。

1.2 仪器与试剂

H-DMEM 培养基:北京索来宝,批号:Lot. No. 20120815;标准胎牛血清:赛默飞世尔生物化学制品(北京)有限公司,批号:NWJ0473;胰蛋白酶:北京索来宝,批号:Lot. No. 20120702。兔多克隆抗 NGF 抗体:1∶500,Chemicon;羊多克隆抗 BDNF 抗体:1∶1000,Chemicon;HRP 标记的兔抗羊 IgG:1∶2000,Protenintech Group;HRP 标记的山羊抗兔 IgG:1∶1000,Protenintech Group;BH-Z 普通光学显微镜:OLYMPUS;Airtech 无菌超净工作台:苏州富泰洁净系统有限公司;低温高速离心机:德国 Heraeus;常温高速离心机:美国 Thermofisher;-40℃低温冰箱:青岛海尔股份有限公司。

2 实验方法

2.1 PRP 制备

SD 大鼠眼眶后静脉取血 2 ml,采用改良的 Appel 法[2]制备富血小板血浆,制备的 PRP 的终浓度为 3%,备用。

2.2 BMSCs 培养

SD 大鼠颈椎脱臼法处死,采用 Kopen G C 的方法[3]培养并纯化出 BMSCs 细胞液,调节细胞浓度为 1×10^6/mL。

2.3 大鼠脊髓损伤模型的建立及给药方法

根据 Nystrom B 方法[4]暴露 T10 段脊髓,打击装置整合于立体定位仪上,将一直径为 3 mm 圆形薄铜垫片(面积 7.075 mm^2,重量 0.1 g)置于 T10 段脊髓表面,以重量为 10 g 的砝码,从 5 cm 高度自由坠落打击该垫片,致伤量为 50 g/cm,造成 T10 段脊髓的冲击伤。在各组损伤段脊髓处分别注入 RPR、BMSCs、BMSCs 与 PRP 复合物,对照组不注入任何物质。造模 8 周后,对损伤段脊髓 HE 染色进行病理学观察。

2.4 Western blot 法检测脊髓中 NGF、BDNF 的表达

取损伤脊髓标本加 100 μL 蛋白裂解液(Tris-HCL,PH7.5;100 mmol·L^{-1} EDTA,0.5 mmol·L^{-1} PMSF)裂解,高速离心 15 min。取上清测蛋白浓度至 5 μg·μL^{-1},SDS-PAGE 蛋白缓冲液按比例加热混合,低温离心 10 min,取上清分装置 -20 ℃备用。制备 SDS-PAGE 胶,4 ℃保存过夜,之后上样进行垂直电泳,用 SDS-PAGE 胶分离后转移到 PVDF 膜上,PVDF 膜用含有 5% 脱脂奶粉的 TBST(Tris-HCL,pH7.4;0.2% Tween-20)溶液封闭 1 h 后,加入相应一抗在 4 ℃共摇床孵育过夜,用 TBST 洗 3 次,每次 5 min,加相应二抗(1∶2 000)在室温共摇床孵育 1 h,经 TBST 洗 3 次,每次 5 min。在暗房内用发光剂发光,显影,定影,分析。

2.5 观察指标

术前 3 d 和术后 8 周,对 SD 大鼠的后肢功能恢复进行 BBB 运动功能评分。术后 8 周,颈椎脱臼处死,取出伤段脊髓组织,HE 染色后,光镜下进行病理学观察。Western blot 法检测脊髓中 NGF、BDFN 蛋白水平。

2.6 统计学方法

采用 SPSS13.0 软件进行统计分析。数据均以 $\bar{x} \pm s$ 表示,经方差齐性检验,方差齐者采用 t 检验。$P < 0.05$ 有统计学意义。

3 结果

3.1 病理学组织观察

光镜下观察,对照组可见神经细胞肿胀,排列疏松,细胞边缘与细胞核模糊不清,神经细胞出血性坏死,神经细胞周围有间隙出现;脊髓坏死区域白细胞浸润,有空腔形成。PRP 组、BMSCs 组的坏死区域减小,炎性细胞浸润减少;神经细胞仍肿胀,细胞边缘与细胞核较对照组清晰,神经细胞无出血性坏死现象,脊髓内空腔较对照组相对减少。PRP + BMSCs 组损伤脊髓的神经细胞细胞边缘与细胞核清楚,水肿减轻,未见神经细胞出血性坏死,空腔较少。

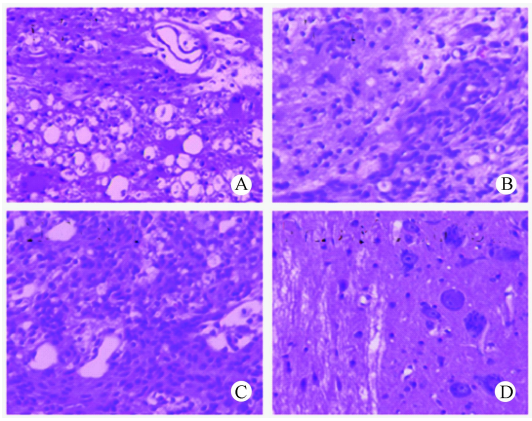

图 1 各治疗组对脊髓损伤大鼠脊髓组织的影响(HE 染色, ×100)

注:A:对照组;B:PRP 组;C:BMSCs 组;D:PRP + BMSCs 组

Fig. 1 Effect of the treatment in each treatment group on spinal cord tissue in rats with spinal cord injuries (HE staining, ×100)

3.2 对脊髓损伤大鼠后肢运动功能的影响

根据 BBB 运动功能评分法,PRP 组、BMSCs 组与 PRP + BMSCs 组的 BBB 分值均比对照组高,且 PRP + BMSCs 组的 BBB 分值最高($P < 0.05$),说明 PRP、BMSCs 可提高脊髓损伤后大鼠的后肢运动功,结果见表 1。

表 1 PRP 和 BMSCs 对脊髓损伤大鼠 BBB 评分变化

Table 1 The changes of BBB scores of rats with spinal cord injuries under the treatment of PRP and BMSCs

组别	分值
对照组	5.2 ± 0.43
PRP 组	6.3 ± 0.56
BMSCs 组	8.6 ± 0.53 *
PRP + BMSCs 组	9.1 ± 0.78 *

注:与空白对照组比较, * $P < 0.05$

3.3 对脊髓中 NGF、BDNF 蛋白表达的影响

结果表明,与对照组相比,各治疗组脊髓的 NGF、BDNF 的蛋白表达水平均有所提高,差异有显著性意义($P < 0.05$)。见表 2,图 2。

表 2 PRP、BMSCs 对脊髓中 NGF、BDNF 蛋白表达的影响($\bar{x} \pm s, n = 10$)

Table 2 Effect of PRP and BMSCs on the expression of NGF and BDNF protein in spinal cord tissue ($\bar{x} \pm s, n = 10$)

组别	NGF	BDFN
对照组	0.22 ± 0.03	0.56 ± 0.08
PRP 组	0.59 ± 0.12 *	0.82 ± 0.06 *
BMSCs 组	0.67 ± 0.09 *	0.89 ± 0.13 *
PRP + BMSCs 组	0.73 ± 0.11 *	0.95 ± 0.12 *

注:与空白对照组比较, * $P < 0.05$

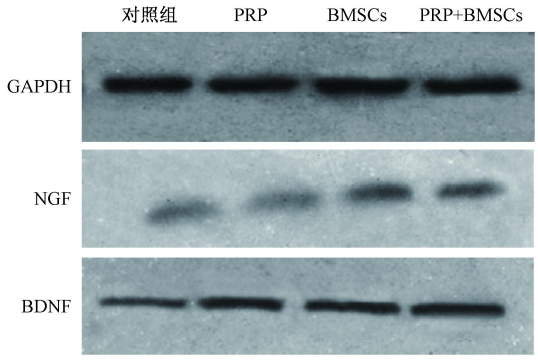

图 2 PRP、BMSCs 对脊髓中 NGF、BDNF 蛋白表达的影响(Western blotting 分析)

Fig. 2 Effect of PRP and BMSCs on the expression of NGF and BDNF protein in spinal cord tissue (Western blotting assay).

3 讨论

目前脊髓损伤的治疗手段难以到达理想的临床效果,脊髓损伤后修复已成为国内外研究的热点。成年人的脊髓神经细胞是体内高度分化的细胞,已经无有丝分裂的能力。在脊髓损伤的修复中主要的修复途径为损伤后残留的神经细胞以终末出芽的方式再生,连接靶细胞,重建神经环路。但这个修复过程中,需要大量的营养因子以提供丰富的神经营养物质。富血小板血浆(PRP)和骨髓间充质干细胞(BMSCs)内含多种神经营养因子,在一定的条件刺激下可分化为神经元细胞[5-7],且 PRP 与 BMSCs 获得方便,对机体损伤小,无免疫排斥反应,对治疗骨头和脊髓损伤等疾病有着广泛良好的前景。

NGF 作为一种神经因子已被证实可促进与维持神经生长、分化的特殊蛋白[8]。神经元以竞争的方式获取微量神经营养因子 NGF,且神经元损伤的情况下,NGF 可对神经元细胞起到保护作用,促进神经元细胞存活。通过提高 NGF 的含量可促进神经元的生长和损伤神经元的修复。

脑源性神经营养因子(brain derived neurophic factor, BDNF)在成年人的脊髓中多分布于灰质[9]。对神经元的存活、分化、生长发育起重要作用,并能防止脊髓损伤后神经元死亡、改善神经元的病理状态、促进受损伤神经元再生及分化等生物效应。Uchida 的研究表明,BDNF 在脊髓损伤中可促进神经元的存活和神经纤维的延长,阻止神经元胞体的萎缩,抑制脊髓损伤后突胶质细胞的延长凋亡[10]。

本实验通过对 HE 染色进行病理学观察,PRP

组、BMSCs 组、PRP + BMSCs 组损伤脊髓的神经细胞都有一定程度的恢复。治疗组的后肢运动功能评分明显高于对照组。与对照组相比,各治疗组脊髓的 NGF、BDNF 的蛋白表达水平均上调。提示脊髓神经元细胞受损后,PRP 与 BMSCs 通过提高脊髓内 NGF、BDNF 的蛋白水平,增加脊髓内神经营养因子的含量,促进神经元样细胞分化,保护损伤区域的神经元细胞,使损伤的脊髓得到修复。

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