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补阳还五汤对脊髓损伤后大鼠大脑皮层运动神经元 IL-17 表达的影响 *

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摘要 目的：通过观察补阳还五汤大鼠急性脊髓损伤后大脑神经元细胞凋亡及 IL-17 的表达的影响，探讨其神经保护机制。**方法：**SD 大鼠 24 只，分成正常组、假手术组、SCI 组、BYHWD 组，通过在 T3-T4 横突间横断右侧半脊髓制备 SCI 模型，分别于手术前 1 d 及术后 1 d、1 w、4 w、8 w 运动 BBB 评分评定大鼠后肢运动功能；术后 8 w 用 Tunnel 法检测神经细胞凋亡、免疫组化法检测大鼠神经细胞 IL-17 的表达。**结果：**BBB 评分比较：SCI 组、BYHWD 组 BBB 评分明显低于正常组与假手术组，差异有统计学意义（P<0.01）；BYHWD 组在术后 4 w、8 w 两个时间点均高于 SCI 组，差异有统计学意义（P<0.05）。细胞凋亡率比较：SCI 组与 BYHWD 组细胞凋亡率均高于正常组与假手术组，且差异有统计学意义（P<0.01）。BYHWD 组凋亡率低于 SCI 组且差异有统计学意义（P<0.01）。IL-17 阳性细胞率比较：SCI 组、BYHWD 组 IL-17 阳性细胞率均显著高于正常组与假手术组（P<0.01）。BYHWD 组 IL-17 阳性细胞率显著低于 SCI 组，差异有统计学意义（P<0.01）。**结论：**SCI 后大脑皮层神经元的凋亡可能与 IL-17 的表达增加有关，补阳还五汤可能通过下调 IL-17 的表达从而减少细胞凋亡。

关键词：补阳还五汤；脊髓损伤；IL-17；细胞凋亡

中图分类号：R651.2；R285.6 **文献标识码：**A **文章编号：**1673-6273(2017)21-4028-04

Effects of Buyang Huanwu Decoction on the Expression of IL-17 in the Cerebral Cortex Neurons of Rats after Spinal Cord Injury*

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ABSTRACT Objective: To observe the effects of Buyang Huanwu Decoction on the apoptosis and expression of IL-17 in cortex neurons in rats after spinal cord injury, in order to explore the possible mechanisms of its neuro protection. **Methods:** 24 SD rats were divided into normal group, sham operation group, SCI group and BYHWD group. SCI model was established in T3-T4 inter transverse the right half of spinal cord transaction. BBB score of rat hind limb motor function were assessed at 1 d before operation and 1 d, 1 W, 4 W, 8 W after operation. After 8 W, Tunnel method was used to detect the apoptosis of nerve cells, and the immunohistochemical staining was used to detect the expression of IL-17 in nerve cells of rats. **Results:** The BBB scores in SCI group and BYHWD group were significantly lower than in the normal group and sham operation group, and the difference was statistically significant (P<0.01). The BBB scores at 4 W and 8 W in BYHWD group were higher than in SCI group, and the difference was statistically significant (P<0.05). The apoptosis rate of SCI group and BYHWD group was higher than that in the normal group and the sham operation group, and the difference was statistically significant (P<0.01). The apoptosis rate of BYHWD group was lower than that of SCI group and the difference was statistically significant (P<0.01). The rate of IL-17 positive cells in SCI group and BYHWD group was significantly higher than in normal group and sham operation group (P<0.01). The rate of IL-17 positive cells in BYHWD group was significantly lower than in SCI group, and the dif- ference was statistically significant (P<0.01). **Conclusion:** The apoptosis in cortical neurons after SCI may be related with the increase of IL-17, and the BYHW Decoction may reduce apoptosis by inhibiting the expression of IL-17.

Key words: Buyang Huanwu decoction; Spinal cord injury; IL-17; Apoptosis

Chinese Library Classification(CLC): R651.2; R285.6 Document code: A

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前言

脊髓损伤（spinal cord injury，SCI）可导致损伤脊髓平面以下严重神经功能缺损，是当前临床治疗的难点[1]。中医认为 SCI 属于督脉受损，经脉淤阻，加之患者多因外伤而大伤元气，导致血淤气虚之证，近年来运用补阳还五汤治疗 SCI 上得到了一定

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效果,本课题组及国内学者在既往的研究中证实补阳还五汤对神经细胞有保护作用,可改善 SCI 损伤大鼠的后肢运动功能[2],其作用机理与调控脊髓组织、脑组织 caspase-3 的表达以及骨成型蛋白受体(BMPR)有关[3,4],但是否可以通过调控 IL-17 的表达尚未明确。本研究通过 SCI 大鼠模型,观察补阳还五汤对大鼠大脑皮层神经细胞 IL-17 表达以及细胞凋亡的影响,为该方治疗 SCI 提供实验依据。

1 材料与方法

1.1 实验动物分组与主要试剂

成年雄性清洁级 SD 大鼠 24 只,体重 200-220 g,湖南中医药大学实验动物中心提供,随机分成补阳还五汤组(BYHWD group,12.82 g 生药 /kg)、正常组 (Normal group)、假手术组(Sham operation group,等体积生理盐水)、SCI 组(SCI group,等体积生理盐水),每组 6 只。各组均腹腔注射给药,给药体积均为 10 mL/kg,1 次 /d,连续 8w。SCI 大鼠模型制备方法参考本课题组文献[21]。TUNLE 细胞凋亡试剂盒(德国 Roche 公司),多克隆兔抗大鼠 IL-17 抗体、生物素化羊抗兔 IgG 和辣根酶标记链酶卵白素(美国 Santa Cruz 公司),DAB(Sigma 公司)。

1.2 BBB 评分法评定大鼠后肢功能

参照 Basso 等 [6] 提出的大鼠 SCI 后后肢功能评判标准(BBB 法),由 3 名经培训的人员分别独立评分后取均值进行统计学处理。

1.3 取材、组织化学及图像数据分析

各组大鼠于设定时间点(术后 8 w)麻醉固定后快速开胸灌注取脑。冠状切后留取组织(视交叉和大脑后极中间段)置于样品托上,PBS 浸没后在自动切片模式下切片(设定振幅 2 mm、厚度 30 μm)。每份标本切 6 片,3 片用于细胞凋亡检测,3 片用于 IL-17 检测。TUNEL 检测细胞凋亡、免疫组化检测 IL-17 的表达等统计学处理均参照课题组文献进行[21]。

2 结果

2.1 大鼠后肢运动功能 BBB 评分结果

从图 1 可以获知:SCI 后各组大鼠均出现大便困难,便质硬结。正常组、假手术组大鼠行走功能未见明显异常。SCI 组、BYHWD 组大鼠损伤平面以下右后肢完全瘫痪。SCI 组、BYHWD 组 BBB 评分明显低于正常组与假手术组,差异有统计学意义(P<0.01);BYHWD 组大鼠 BBB 评分在术后 1 d、1 w 两个时间点与 SCI 组相比,差异无统计学意义(P>0.05);术后 4 w、8 w,BYHWD 组大鼠评分高于 SCI 组,差异有统计学意义(P<0.05)。

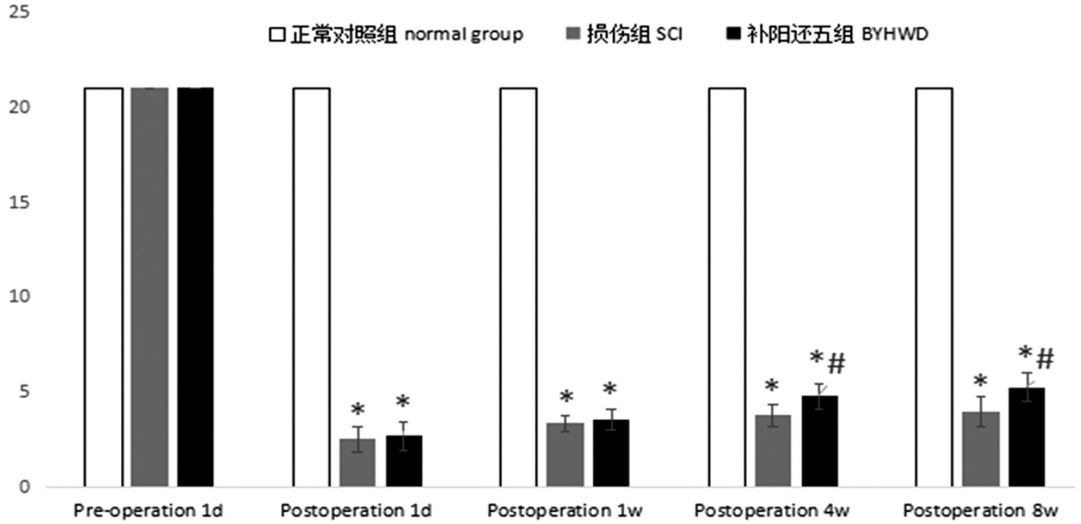

图 1 各组大鼠不同时间点后肢 BBB 评分比较($\bar{x}\pm$ s)

Fig.1 Comparison of BBB scores of each rat group at different time points (Mean ± SD, n=6)

注:与正常组、假手术组相比,*P<0.01;与 SCI 组相比,#P<0.05。

Note:* : vs normal group and sham operation group, P<0.01; #: vs SCI group, P<0.05.

2.2 术后 8 w 大鼠大脑皮层细胞凋亡情况

从表 1 可以获知:正常组与假手术组大鼠大脑运动皮质 M1 区可见少量 TUNEL 阳性细胞;SCI 组与 BYHWD 组可见较多 TUNEL 阳性细胞(图 2),且与正常组、假手术组相比,细胞凋亡率有统计学意义(P<0.01)。BYHWD 组与 SCI 组比较:BYHWD 组低于 SCI 组且差异有显著统计学意义(P<0.01)。

2.3 术后 8 w 大鼠大脑皮层细胞 IL-17 表达情况

从表 2 可以获知:正常组与假手术组大脑皮层细胞可见少量散在的 IL-17 标记阳性神经元;SCI 组、BYHWD 组大鼠可见较多 IL-17 标记阳性神经元(图 3)。SCI 组、BYHWD 组 IL-17 阳性细胞率显著高于正常组与假手术组(P<0.01)。BYHWD 组与 SCI 组比较:BYHWD 组低于 SCI 组且差异有显著统计学意义(P<0.01)。

表 1 术后 8w 各组大鼠神经细胞凋亡率比较($\bar{x}\pm$ s,%)

Table 1 Comparison of the apoptosis rate of each rat group at 8W post-operation (Mean ± SD, %)

Groups	n	8 w post-operation
Normal group	6	1.37± 0.55
Sham operation group	6	1.78± 0.63
SCI group	6	30.25± 7.95*
BYHWD group	6	10.25± 3.234*△

Note: * : vs normal group and sham operation group, P<0.01; △ : vs SCI group, P<0.01.

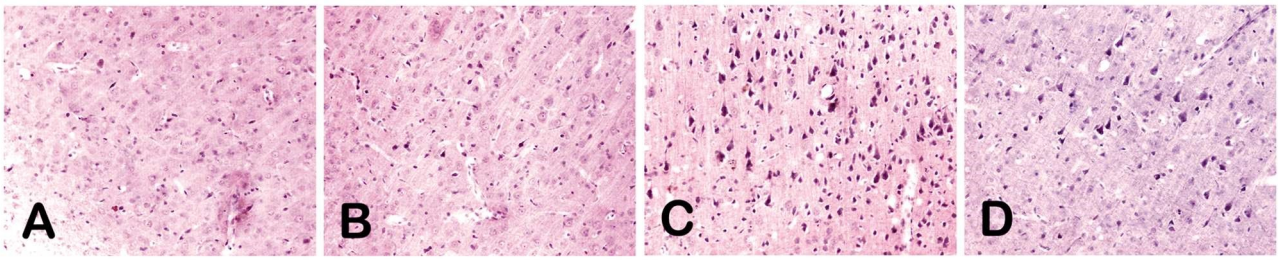

图 2 术后 8w TUNEL 染色显示细胞凋亡情况(× 200 倍)

注:正常组(A)、假手术组(B)偶见神经凋亡细胞,SCI 组(C)可见大量神经凋亡细胞,BYHWD 组(D)神经凋亡细胞数量较 SCI 组(C)明显减少。

Fig. 2 The apoptosis of cortex neuron expressed by TUNEL at 8W post-operation (× 200 times)

Note: Normal group (A): Sham operation group (B): rare neuronal apoptosis; SCI group (C): large number of neuron apoptosis; BYHWD group (D): the number of neuron apoptosis decreased apparently as compared with SCI group.

表 2 术后 8w 各组大鼠 IL-17 阳性细胞率比较($\bar{x}\pm$ s,%)

Table 2 Comparison of the rate of IL-17 positive cell in each group at 8W post-operation (Mean ± SD, %)

Groups	n	8 w post-operation
Normal group	6	3.37± 0.45
Sham operation group	6	2.88± 0.51
SCI group	6	20.15± 6.89*
BYHWD group	6	8.98± 4.29*△

Note:*: vs. normal group and sham operation group, P<0.01, △ :vs. SCI group, P<0.01.

3 讨论

继发性损伤是脊髓原发性损伤后由分子和细胞介导的复杂的病理过程,可逆性且可被调控[7]。细胞凋亡是导致脊髓继发性损伤的关键因素。中医理论认为,脊髓属督脉,上络于脑,故脊髓损伤可影响到脑,这点也已被临床和动物实验证实。Hou JM 等[8]发现 SCI 患者早期即可见大脑躯体感觉皮质(S1 区)萎缩程度加重;Wrigley 等[9]发现 SCI 患者大脑运动皮质(M1 区)的灰质容积较正常人明显减少。实验也证实,SCI 后相应大脑运动神经元会产生继发性病理改变(细胞凋亡、死亡等)[10,11],目前推测与炎性细胞因子(IL-17、IL-10 等)有关[12-15],但具体的机理仍不明确。

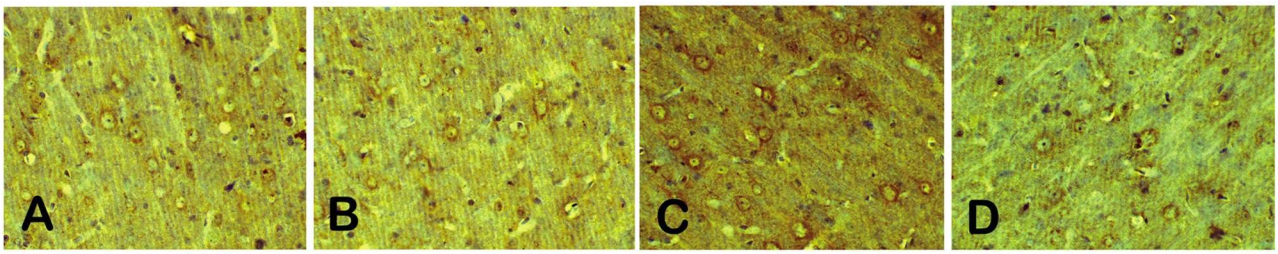

图 3 术后 8w 免疫组化显示 IL-17 阳性细胞(胞浆棕黄色者)情况(× 400 倍)

注:正常组(A)、假手术组(B)偶见 IL-17 阳性细胞,SCI 组(C)可见大量 IL-17 阳性细胞,BYHWD 组(D)IL-17 阳性细胞数量较 SCI 组(C)明显减少。

Fig. 3 Immunohistochemistry of IL-17 in cortex cells of spinal cord of rats at 8W post-operation (× 400)

Note: normal group(A), sham operation group(B): rare IL-17 positive cells; SCI group(C): large number of IL-17 positive cells; BYHWD group(D): the number of IL-17 cells in decreased apparently as compared with SCI group.

IL-17 作为一种强大的前炎性细胞因子,可通过多个信号转导通路(TRAF6、NIK、IKK-α、NF-κB 等)诱导细胞释放前炎性因子,动员中性粒细胞释放一系列细因子而发挥作用。大量实验研究已经证实,SCI 后脊髓损伤部位中性粒细胞、巨噬细胞、淋巴细胞、小胶质细胞的激活、活化均可产生 IL-17。IL-17 可以诱导促炎因子(IL-1β、IL-6、TNF-α)的表达,还可以与 IL-1β、TNF-α 等炎性因子协同而加强其致炎效应[16]。也有研究表明,IL-17 极其诱导的机体炎症反应是导致脑缺血、自身免疫性脑脊髓炎等中枢神经系统疾病继发性损伤的重要环节,其在中枢神经系统中主要表达于 T 淋巴细胞、神经胶质细胞以及神经元细胞[17,18]。同时,SCI 归属于"血瘀气虚"证范畴[19],治疗上以益气补血、祛瘀通络为原则。补阳还五汤原本用于治疗中风,近来临床上用于治疗 SCI 亦取得良好疗效。本课题组前期的研究发现,SCI 后大脑相应皮层神经元会发生萎缩甚至坏死,而这些神经元可以通过黄芪注射液、补阳还五汤的干预而得到保护[20,21]。

根据本实验的结果,我们推测,SCI 后大脑皮层神经元的凋亡增加可能与 IL-17 的表达增加有关,补阳还五汤可以通过下调 IL-17 的表达从而减少神经细胞的凋亡。但究竟通过何种机制来调控 IL-17 的表达,仍需进一步研究。

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