

·基础研究·

针刺百会、曲鬓穴对急性期脑出血大鼠的影响及其作用机制研究

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摘要 **目的** 观察针刺百会、曲鬓穴对急性期脑出血(ICH)大鼠白细胞分化抗原36(CD36)、Toll样受体4(TLR4)表达的影响,探讨针刺治疗脑出血的作用机制。**方法** 选择144只Wistar雄性大鼠,采用随机数字表法分为假手术组、模型组、针刺组、抑制剂组4组,每组36只,每组按1、3、7 d时间点再分为3个亚组,每组12只。采用立体定位自体血注入法建立ICH大鼠模型。模型组仅接受ICH模型制备,不进行任何治疗;假手术组接受类似模型组各项手术操作,但不进行注血制作;抑制剂组造模后6 h,腹腔注射TLR4抑制剂TAK242,3 mg/kg,1次/d,连续5 d;造模12 h后,针刺组各亚组开始接受针刺治疗,穴位选择百会穴(顶骨正中)、右侧曲鬓穴,采用透刺方法,进针深度20 mm,以100 r/min小幅度捻转,持续捻转2 min,每间隔5 min捻针1次,共留针30 min,期间捻转3次,1次/d,针刺组各亚组分别治疗1、3、7 d。分别于治疗后第1、3、7天,采用改良神经功能缺损评分(mNSS)评估大鼠神经功能;检测脑组织血肿体积;采用Western blot法检测脑组织CD36、TLR4蛋白表达水平;采用免疫荧光法观察CD36、TLR4在星形胶质细胞中的表达。**结果** ① mNSS评分:与假手术组同一时间点比较,模型组、针刺组、抑制剂组治疗后1、3、7 d mNSS评分均明显升高($P < 0.05$);与模型组同一时间点比较,针刺组、抑制剂组治疗后1、3、7 d mNSS评分均明显降低($P < 0.05$);与针刺组同一时间点比较,抑制剂组治疗后1 d mNSS评分明显降低($P < 0.05$)。② 血肿体积:与模型组同一时间点比较,针刺组、抑制剂组治疗后3、7 d脑血肿体积均明显降低,抑制剂组治疗后1、3、7 d脑血肿体积明显降低($P < 0.05$);与针刺组同一时间点比较,抑制剂组治疗后1、3、7 d脑血肿体积明显降低($P < 0.05$)。③ CD36、TLR4蛋白表达水平:与假手术组同一时间点比较,模型组、针刺组、抑制剂组治疗后1、3、7 d CD36、TLR4蛋白表达水平均明显升高($P < 0.05$);与模型组同一时间点比较,针刺组、抑制剂组治疗后1、3、7 d CD36蛋白表达水平均明显升高($P < 0.05$),针刺组、抑制剂组治疗后3、7 d TLR4蛋白表达水平均明显降低($P < 0.05$);与针刺组比较,抑制剂组治疗后1、3、7 d CD36蛋白表达水平均明显升高,TLR4蛋白表达水平均明显降低($P < 0.05$)。④ CD36、TLR4在GFAP中的表达水平:假手术组大鼠脑组织内可见少量CD36、TLR4表达。与假手术组同一时间点比较,模型组治疗后1、3、7 d CD36、TLR4在GFAP表达均明显增加($P < 0.05$);与模型组同一时间点比较,抑制剂组、针刺组治疗后1、3、7 d CD36在GFAP表达明显增加,TLR4在GFAP表达明显降低($P < 0.05$)。**结论** 针刺百会、曲鬓穴可以改善急性期ICH大鼠神经功能,减轻脑出血血肿体积,可能与促进CD36蛋白表达、抑制TLR4蛋白表达有关。

关键词 脑出血;针刺;百会穴;曲鬓穴;神经功能;白细胞分化抗原36;Toll样受体4

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脑出血(cerebral hemorrhage, ICH)是急性脑血管病中病死率最高的疾病,占急性脑血管病的20%~30%,急性期ICH病死率高达40%^[1]。有研究显示,近20年全球脑出血发病率上升47%,严重影响人们健康^[2]。脑出血后小胶质细胞及星形胶质细胞活化,血肿压迫及炎性浸润会破坏脑组织。脑出血后脑损伤主要因素是脑出血后血肿对周围脑组织的直接压迫作用,出血部位和血肿周围半暗带区缺血、缺氧引发的继发性脑损伤^[3]。此外,神经炎症反应和凝块成分的释放也会引起继发性脑损伤。有研究显示,星形胶质细胞、脑出血血肿吸收和炎症反应三者间具有紧密的联系^[4-8]。白细胞分化抗原36(recombinant cluster of differentiation 36, CD36)作为清道夫Ⅱ型受体具有强大的吞噬功能,受到学者们的广泛关注。有研究显示,CD36可能介导了脑出血血肿内源性吸收^[9-10],可减轻神经细胞损伤,改善神经功能恢复。Toll样受体(Toll-like receptors, TLRs)是一类保守的天然免疫受体,能感知入侵的微生物病原体,并在先天免疫和获得性免疫的激活中发挥桥梁作用^[11]。Toll样受体4(Toll-like receptor 4, TLR4)作为哺乳动物TLRs第1个成员,在免疫应答、炎症反应以及细胞活化信号的转导等多个过程中发挥重要作用^[12-13]。在脑出血后,CD36通过吞噬血肿,减轻细胞损伤,有助于神经功能的恢复;TLR4激活会引发炎症反应,加剧细胞损伤,影响神经功能恢复。CD36和TLR4在脑出血后的神经功能恢复中起到了相互制衡的作用,这对于控制炎症反应和恢复神经功能至关重要。因此,本研究猜测调

控CD36和TLR4的表达和功能,可能有助于改善脑出血后的神经功能恢复。

本团队前期研究表明,针刺可以减轻脑出血组织病理结构破坏程度和炎症反应^[14-15],抑制脑出血大鼠TLR4信号激活和下游信号分子骨髓分化初级反应蛋白88(myeloid differentiation factor 88, MyD88)以及核因子- κ B(nuclear factor kappa-B, NF- κ B)炎症因子的活化,降低肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)、白细胞介素-1 β (interleukin 1 β , IL-1 β)、白细胞介素-6(interleukin 6, IL-6)经典炎症因子表达,降低脑出血后炎症继发性损害,发挥脑保护作用^[16-17]。但从血肿吸收和炎症反应相关的角度探讨针刺对脑保护作用的相关研究较少。本研究采用针刺百会、曲鬓穴干预脑出血大鼠,观察头针对ICH大鼠血肿组织CD36、TLR4蛋白表达及星形胶质细胞的影响,以期能为针刺治疗脑出血提供理论支持。

1 材料与方法

1.1 实验动物

实验动物由吉林大学白求恩医学院动物实验中心提供SPF级Wistar雄性大鼠144只,8~9周龄,体质量280~320 g[实验动物生产许可证号:SCXK(吉)2013-0004]。所有动物饲养在温度(22±3)℃、相对湿度60%~65%、12/12 h明暗变化条件下,大鼠自由摄食进水。实验遵循科技部颁布《关于善待实验动物的指导性意见》相关规定。

1.2 主要试剂及仪器

主要试剂及仪器见表1。

表1 主要试剂及仪器

Table 1 Main reagents and instruments

试剂及仪器	型号	生产厂家
CD36多克隆抗体	A1470	武汉爱博泰克生物科技有限公司
TLR4多克隆抗体	A5258	武汉爱博泰克生物科技有限公司
Glial Fibrillary Acidic Protein单克隆抗体	Sc-33673	美国圣克鲁斯生物技术公司
FITC标记山羊抗小鼠IgG(绿光)	A5608	上海碧云天生物技术有限公司
Cy3标记山羊抗兔IgG(红光)	A0516	上海碧云天生物技术有限公司
立体定位仪	ST-5ND-C	中国成都仪器厂

1.3 动物模型制备和分组

1.3.1 实验动物分组 采用随机数字表法将144只Wistar大鼠分为模型组、假手术组、抑制剂组(TAK242组)和针刺组,每组36只。每组按1、3、7 d时间点再分为3个亚组,每组12只。

1.3.2 实验动物模型制备 参考文献[18]采用立

体定位自体血注入法制作ICH实验动物模型。使用1%戊巴比妥钠(50 mg/kg)腹腔注射将大鼠麻醉,麻醉后将大鼠头部固定在立体定位仪上,备皮局部消毒,头顶正中切口约1 cm,使用骨膜剥离器将骨膜剥离开,充分暴露出前囟及冠状缝位置,定位前囟点(Bregma点),在Bregma点右旁开3.5 mm,后0.2 mm

定位,使用牙科钻钻出直径约为1.0 mm圆孔,深度到达硬脑膜表面,用酒精消毒大鼠的尾端(5 cm),距尾端4 cm左右处剪断鼠尾,使用器皿收集血液,微量注射器抽取血液50 μ L,再将微量注射器固定在立体定位仪上,微量注射器针头沿牙科钻孔进针6 mm,缓慢将血液以20 μ L/min速度推进大鼠尾壳核,停留5 min后缓慢拔出微量注射器,头顶局部消毒,用骨蜡封闭颅骨伤口,缝合头皮,局部皮肤采用碘伏消毒,在注血和留针期间消毒包扎鼠尾伤口。造模完成至大鼠清醒后(约术后6 h),采用Berderson评分法^[19]确定造模是否成功,评分1~3分大鼠纳入实验。

1.4 治疗方法

1.4.1 模型组 仅接受ICH模型制备,不进行任何治疗。

1.4.2 假手术组 接受类似模型组各项手术操作,但不进行注血制作。

1.4.3 抑制剂组 造模后6 h,腹腔注射TLR4抑制剂TAK242,3 mg/kg,1次/d,连续5 d^[20]。

1.4.4 针刺组 造模12 h后,针刺组各亚组开始接受针刺治疗。参照《实验针灸学》^[21]选取大鼠百会穴(顶骨正中)、右侧曲鬓穴(右眶外缘与右外耳门连线后2/3),采用透刺方法,手持针灸针(0.30 mm \times 25 mm,贵州安迪药械有限公司)从百会穴向曲鬓穴快速平刺进针(与头皮成15°进针),进针深度20 mm,以100 r/min小幅度捻转,持续捻转2 min,每间隔5 min捻针1次,共留针30 min,期间捻转3次,1次/d,针刺组各亚组分别治疗1、3、7 d。

1.5 观察指标

1.5.1 神经功能评分 分别于治疗后第1、3、7天,选择12只大鼠采用改良神经功能缺损评分(modified neurological severity score, mNSS)评估大鼠神经功能^[22-23]。mNSS评分总分18分。1~6分为轻度损伤;7~12分为中度损伤;13~18分为重度损伤。

1.5.2 脑组织血肿体积 经mNSS评分后,每组采用随机数字表法选取6只大鼠,麻醉后,颈椎脱臼处死、断头、取脑组织,4%多聚甲醛溶液固定72 h,通过高速冷冻切片机、以注射针眼处冠状位向前后各均匀切出4个1 mm厚度(t)的脑片,按顺序平摊后,应用Image Pro Plus 5.0软件测量脑出血面积(A_n),然后计算血肿体积(V)^[24]。

$$\text{血肿体积}(V) = (A_1 + A_2 + A_3 \cdots + A_n) \times t$$

1.5.3 Western blot法检测脑组织CD36、TLR4蛋白表达水平 从-80 $^{\circ}$ C冰箱取4组大鼠脑组织,将脑组

织匀浆离心后取上清液,采用BCA法测定蛋白量。将30 μ L处理后的蛋白样品采用12%十二烷基硫酸钠-聚丙烯酰胺(sodium dodecyl sulfate-polyacrylamide gel electrophoresis, SDS-PAGE)凝胶电泳转移至聚偏氟乙烯(polyvinylidene fluoride, PVDF)膜,以标准蛋白Maker为参照,5%脱脂奶粉封闭,相应条带分别加入CD36(1:500)、TLR4(1:4 000),4 $^{\circ}$ C孵育过夜。一抗孵育完毕后,取出PVDF膜进行二抗孵育CD36(1:5 000),TLR4(1:5 000),37 $^{\circ}$ C下孵育45 min。孵育并显色完成后,将硝酸纤维素膜移至蒸馏水中终止显色。用滤纸将膜吸干,避光放置20 min后,将膜置于扫描仪中扫描,用凝胶图像处理系统(Gel-Pro-Analyzer软件)分析目标条带的吸光度值(optical density, OD)。

1.5.4 免疫荧光法观察CD36、TLR4在星形胶质细胞中的表达 采用多聚螯合物酶组化(power vision, PV)双重染色法,进行CD36、TLR4和标记神经胶质细胞原纤维酸性蛋白(glial fibrillary acidic protein, GFAP)双标,将脑组织进行包埋、切片,将切片架分别浸于I级和II级二甲苯中脱蜡各15 min。取出切片,依次浸入95%、85%、75%乙醇各1 min;继续浸泡在磷酸盐缓冲溶液(phosphate-buffered saline, PBS)中5 min \times 3次。置于煮沸抗原修复液中,低火修复10 min;滴加山羊血清至完全覆盖组织,湿盒内室温封闭15 min。滴加抗体CD36(1:200)、TLR4(1:200),4 $^{\circ}$ C孵育过夜。孵育完毕后,滴加荧光二抗,二者均用PBS 1:200稀释(以下操作注意避光),滴加完全覆盖组织,室温孵育90 min。复染及浸泡结束后,胶头滴管滴加半滴抗荧光淬灭剂,盖玻片封片。于荧光显微镜下观察染色效果(标尺长度50 μ m)。

1.6 统计学方法

采用SPSS 26.0统计软件进行数据分析。计量资料服从正态分布以($\bar{x} \pm s$)表示,组间比较采用单因素方差分析,两两比较采用LSD- t 检验。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 4组mNSS评分比较

与假手术组同一时间点比较,模型组、针刺组、抑制剂组治疗后1、3、7 d mNSS评分均明显升高($P < 0.05$);与模型组同一时间点比较,针刺组、抑制剂组治疗后1、3、7 d mNSS评分均明显降低($P < 0.05$);与针刺组同一时间点比较,抑制剂组治疗后1 d mNSS评分明显降低($P < 0.05$)。见表2。

表2 4组mNSS评分比较($\bar{x}\pm s$)
Table 2 Comparison of mNSS score in four groups ($\bar{x}\pm s$)

分
Scores

组别	<i>n</i>	治疗后1 d	治疗后3 d	治疗后7 d
假手术组	12	0.67±0.52	0.50±0.55	0.50±0.55
模型组	12	11.33±0.52 ¹⁾	8.33±0.52 ¹⁾	7.12±0.41 ¹⁾
针刺组	12	6.67±0.52 ¹⁾²⁾	4.83±0.41 ¹⁾²⁾	4.50±0.55 ¹⁾²⁾
抑制剂组	12	5.50±0.55 ¹⁾²⁾³⁾	4.67±0.52 ¹⁾²⁾	4.33±0.52 ¹⁾²⁾

注:与假手术组同一时间点比较,1) $P<0.05$;与模型组同一时间点比较,2) $P<0.05$;与针刺组同一时间点比较,3) $P<0.05$ 。
Note: Compared with the sham operation group at the same time, 1) $P<0.05$; compared with the model group at the same time, 2) $P<0.05$; compared with the acupuncture group at the same time, 3) $P<0.05$.

2.2 4组脑血肿体积比较

与模型组同一时间点比较,针刺组、抑制剂组治疗后3、7 d脑血肿体积均明显降低($P<0.05$),抑

制剂组治疗后1、3、7 d脑血肿体积明显降低($P<0.05$);与针刺组同一时间点比较,抑制剂组治疗后1、3、7 d脑血肿体积明显降低($P<0.05$)。见表3。

表3 4组脑血肿体积比较($\bar{x}\pm s$)
Table 3 Comparison of cerebral hematoma volume in four groups ($\bar{x}\pm s$)

mm³
mm³

组别	<i>n</i>	治疗后1 d	治疗后3 d	治疗后7 d
假手术组	6	—	—	—
模型组	6	19.44±1.09	36.73±1.28	17.26±0.77
针刺组	6	18.79±1.46	33.17±1.19 ¹⁾	16.32±0.85 ¹⁾
抑制剂组	6	17.67±1.23 ¹⁾²⁾	31.45±1.32 ¹⁾²⁾	15.45±0.83 ¹⁾²⁾

注:与模型组同一时间点比较,1) $P<0.05$;与针刺组同一时间点比较,2) $P<0.05$ 。

Note: Compared with the model group at the same time, 1) $P<0.05$; compared with the acupuncture group at the same time, 2) $P<0.05$.

2.3 4组脑组织CD36、TLR4蛋白表达水平比较

与假手术组同一时间点比较,模型组、针刺组、抑制剂组治疗后1、3、7 d CD36、TLR4蛋白表达水平均明显升高($P<0.05$);与模型组同一时间点比较,针刺组、抑制剂组治疗后1、3、7 d CD36蛋白表达水平均明显升高($P<0.05$),针刺组、抑制剂组治疗后3、7 d TLR4蛋白表达水平均明显降低($P<0.05$);与针刺组比较,抑制剂组治疗后1、3、7 d CD36蛋白表达水平均明显升高($P<0.05$),TLR4蛋白表达水平均明显降低($P<0.05$)。见表4、图1。

2.4 4组脑组织CD36、TLR4在GFAP中的表达水平比较

从免疫荧光图中可以看见假手术组大鼠脑组织内少量CD36、TLR4表达。与假手术组同一时间点比较,模型组治疗后1、3、7 d CD36、TLR4在GFAP表达均明显增加($P<0.05$);与模型组同一时间点比较,抑制剂组、针刺组治疗后1、3、7 d CD36在GFAP表达明显增加,TLR4在GFAP表达明显降低($P<0.05$)。见图2、图3。

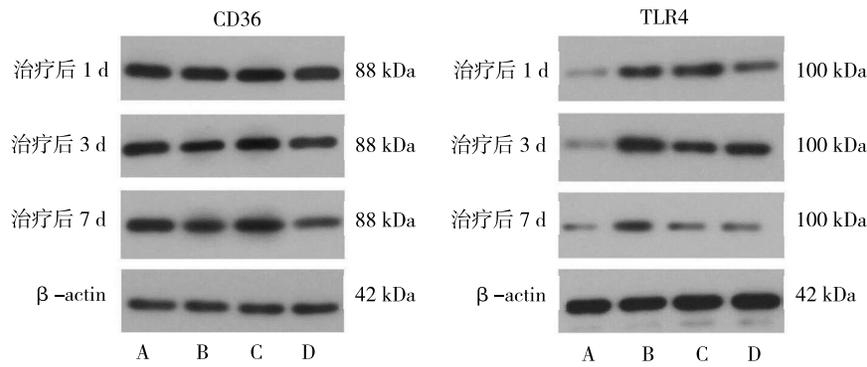
表4 4组脑组织CD36、TLR4蛋白表达水平比较($\bar{x}\pm s$)

Table 4 Comparison of CD36 and TLR4 protein expression level in brain tissue in four groups ($\bar{x}\pm s$)

组别	<i>n</i>	时间	CD36	TLR4
假手术组	6	治疗后1 d	0.90±0.01	0.95±0.09
		治疗后3 d	0.88±0.02	0.92±0.06
		治疗后7 d	0.90±0.02	0.92±0.04
模型组	6	治疗后1 d	1.00±0.03 ¹⁾	2.18±0.04 ¹⁾
		治疗后3 d	1.12±0.02 ¹⁾	3.23±0.06 ¹⁾
		治疗后7 d	1.05±0.04 ¹⁾	3.01±0.05 ¹⁾
针刺组	6	治疗后1 d	1.07±0.03 ¹⁾²⁾	2.13±0.05 ¹⁾
		治疗后3 d	1.20±0.02 ¹⁾²⁾	2.48±0.06 ¹⁾²⁾
		治疗后7 d	1.13±0.02 ¹⁾²⁾	1.74±0.07 ¹⁾²⁾
抑制剂组	6	治疗后1 d	1.10±0.05 ¹⁾²⁾³⁾	1.67±0.04 ¹⁾²⁾³⁾
		治疗后3 d	1.33±0.05 ¹⁾²⁾³⁾	1.89±0.08 ¹⁾²⁾³⁾
		治疗后7 d	1.17±0.02 ¹⁾²⁾³⁾	1.54±0.05 ¹⁾²⁾³⁾

注:与假手术组同一时间点比较,1) $P<0.05$;与模型组同一时间点比较,2) $P<0.05$;与针刺组同一时间点比较,3) $P<0.05$ 。

Note: Compared with the sham operation group at the same time, 1) $P<0.05$; compared with the model group at the same time, 2) $P<0.05$; compared with the acupuncture group at the same time, 3) $P<0.05$.



注:A为假手术组;B为模型组;C为抑制剂组;D为针刺组。

Note: A is the sham group; B is the model group; C is the inhibitor group; D is the acupuncture group.

图1 4组脑组织CD36、TLR4蛋白条带图

Figure 1 Protein band figure of CD36 and TLR4 in four groups

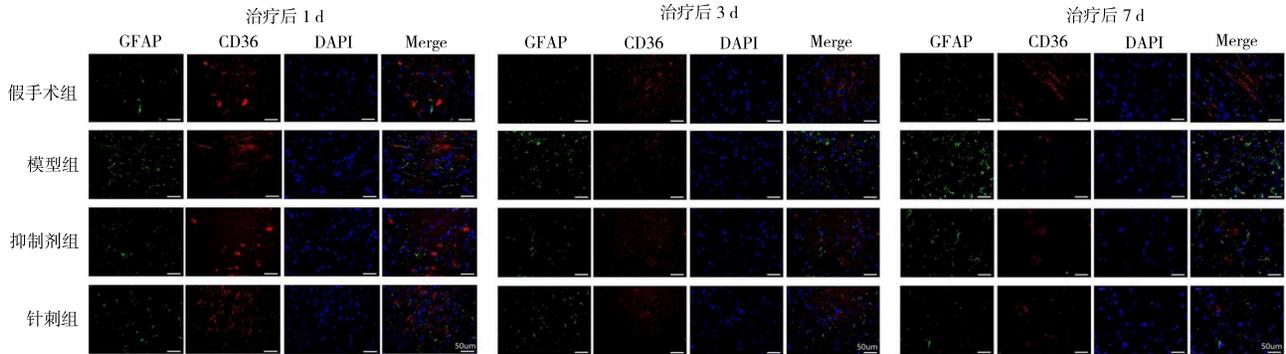


图2 4组脑组织CD36在GFAP中的表达水平比较

Figure 2 Comparison of CD36 expression level in GFAP of brain tissue in four groups

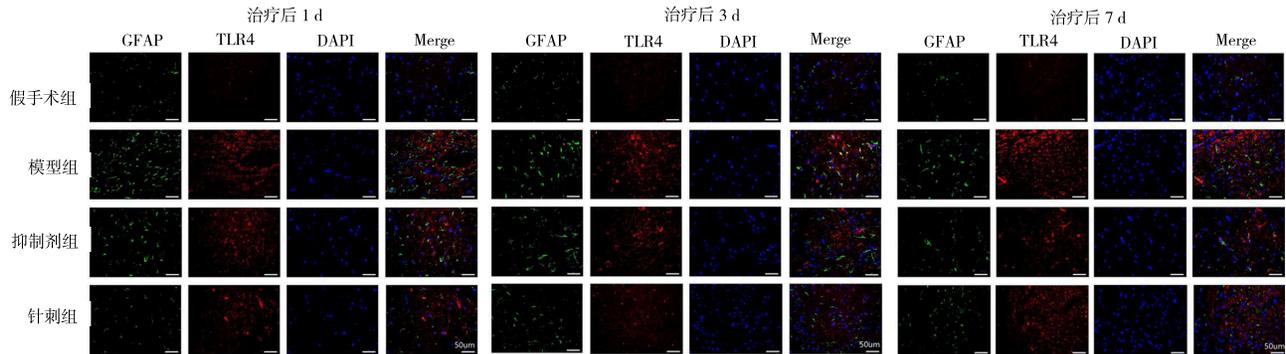


图3 4组脑组织TLR4在GFAP中的表达水平比较

Figure 3 Comparison of TLR4 expression level in GFAP of brain tissue in four groups

3 讨论

脑出血后神经功能损伤原因可分为直接损伤及继发性损伤,出血造成的血肿对脑组织直接压迫为直接损伤,而因血肿代谢产物引发的一系列反应为继发性损伤,二者均可加重脑水肿及神经损伤^[25-26]。其中直接压迫是由脑出血发生后血液积聚并压缩周围的脑组织,导致组织损伤和神经元死亡^[27-28]。脑出血的病理过程较为复杂,是多种途径

损害的综合病理过程,包括血肿毒性、高代谢损伤、兴奋毒性、氧化应激和炎症损伤^[29-32]。CD36和TLR4在脑出血的血肿吸收和炎症反应中相互影响。头针作为治疗脑卒中重要手段之一,临床中可以明显提高脑卒中患者的肢体功能^[33-36],但其作用机理尚不明确。因此,本实验采用针刺百会透曲鬓穴干预脑出血大鼠,观察血肿组织CD36、TLR4蛋白表达及其在星形胶质细胞中的表达水平,以期针刺治

疗脑出血提供参考。

3.1 针刺百会、曲鬓穴可改善急性期ICH大鼠神经功能和脑组织血肿体积

本研究结果显示,与模型组同一时间点比较,针刺组治疗后1、3、7 d mNSS评分均明显降低,治疗后3、7 d 脑血肿体积均明显降低,提示针刺百会、曲鬓穴可改善急性期ICH大鼠神经功能和脑组织血肿体积。可能与以下原因有关:①头针是在中国传统针灸学基础上,结合大脑皮层功能定位原理及生物全息理论规律的基础上形成。从电生理角度分析,将头部视作一个容积导体。针刺头部腧穴产生的刺激,通过头部导体的作用将针刺的生物电效应投射到大脑皮层,使大脑皮层的细胞兴奋活跃,纠正抑制性泛化,脑皮质功能逐渐恢复,使得受出血或血肿压迫抑制的神经细胞接受刺激而活跃,促进脑功能恢复。此外,脑出血属于中风,病位在脑,针刺头部腧穴,使针刺的经气通过经络传导将刺激作用于脑,达到治疗疾病的目的^[37]。百会穴意为百脉于此交会,隶属于督脉。曲鬓穴隶属于足少阳胆经。有研究显示,针刺百会穴和曲鬓穴可治疗中风^[38]。针刺百会穴和曲鬓穴可通过促进局部血液循环,改善脑出血患者脑组织血液灌注,有效促进血肿吸收和清除,从而减少血肿体积和脑组织损伤。②mNSS是一种评估动物特别是啮齿类动物神经损伤严重程度和运动、感觉、平衡及反射等功能恢复情况的综合评分系统,其评分标准包括多个测试,整体分数的降低可能反映了大鼠运动协调能力的改善、感觉缺失的减轻、平衡能力的增强以及更好的反射功能。此外,mNSS评分改善还可能意味着在脑出血的病理过程中,如炎症反应、细胞凋亡和脑水肿等可能有所减轻。这可能与针刺百会穴和曲鬓穴会激活神经保护机制,减少神经损伤,促进神经再生有关^[22-23]。③脑出血后血肿会导致周围脑组织损伤和神经功能丧失。血肿体积的大小直接关系到损伤的严重程度,较大血肿可能会导致更高颅内压,进而压迫脑组织,影响脑内血流,甚至可能导致脑疝发生,这些都可能加剧脑损伤。针刺百会、曲鬓穴后,ICH模型大鼠血肿体积更小,提示该治疗方法可有效促进血肿吸收和提高血肿清除能力。

3.2 针刺百会、曲鬓穴改善急性期ICH大鼠神经功能和脑组织血肿体积可能与调节CD36、TLR4蛋白表达有关

本研究结果显示,与模型组同一时间点比较,针刺组治疗后1、3、7 d CD36蛋白表达明显增加,治疗后3、7 d TLR4蛋白表达明显降低,提示针刺百会、

曲鬓穴改善急性期ICH大鼠神经功能和脑组织血肿体积可能与调节CD36、TLR4蛋白表达有关。

3.2.1 促进血肿清除 CD36作为B类清道夫受体家族的一员,可以在多种细胞类型中表达^[28-29,39-40],可识别多种外源性和内源性的有害物质,参与介导人体多种生理、病理过程^[27,41-42]。有研究表明,CD36是脑出血后血肿清除的关键调节因子^[43-45],CD36可能在促进血肿吸收中具有重要作用,进而影响患者的神经功能恢复^[46]。针刺可能会增加CD36蛋白表达,增强巨噬细胞对血肿中红细胞残骸的吞噬作用,加速血肿清除,减少血肿体积,降低对周围脑组织的压迫,从而改善神经功能。

3.2.2 减轻炎症反应 TLRs家族是经典的炎症反应标志物,介导多种免疫通路,其家族中的多种受体均参与中枢神经系统的多种疾病^[47-49]。脑出血后血肿周围脑组织TLR4 mRNA表达明显增多,于48 h达到高峰,直至术后7 d仍有表达,这与脑出血后周围组织水肿呈现出的结果趋势相同,因此,脑出血后血肿周围组织内TLR4高度表达可能与血脑屏障的破坏以及脑水肿相关^[50-51]。针刺后,ICH模型大鼠TLR4表达降低,这可以减少细胞因子和炎症介质的释放,减轻脑组织的炎症损伤,从而有利于神经功能的恢复。这与有研究显示TLR4信号通路可能参与负调控CD36的表达,进而影响脑出血血肿吸收的结果一致^[52-54]。

3.2.3 抑制GFAP活化 GFAP是星形胶质细胞活化的标志物,针刺百会、曲鬓穴可抑制急性期ICH大鼠星形胶质细胞的活化,降低GFAP表达,这有助于维持神经环境稳定,减少继发性损伤。针刺百会、曲鬓穴可使GFAP表达降低,同时TLR4表达也会受到抑制,促进神经修复相关因子(如神经生长因子)的释放,从而促进损伤神经元的再生和修复。

3.2.4 改善血脑屏障功能 本研究结果显示,针刺组和抑制剂组大鼠治疗后1、3、7 d时CD36蛋白表达明显升高,提示针刺百会、曲鬓穴可改善急性期ICH大鼠血脑屏障功能,减少血脑屏障的通透性,防止进一步损伤脑组织,促进血肿吸收和减少炎症。CD36表达的效应可能是针刺加速大鼠脑出血恢复的重要机制之一。针刺治疗ICH模型大鼠后,CD36表达增加可能有助于清除血肿和细胞残骸,促进炎症清除和组织修复。此外,TLR4是免疫系统中识别病原体和损伤信号的受体,其激活可引发炎症反应。ICH模型大鼠TLR4的过度表达可能与神经炎症和神经功能损伤有关。针刺百会、曲鬓穴后3、7 d,TLR4蛋白的表达明显降低,与抑制剂组结果类似,

提示针刺可能通过抑制TLR4激活以减轻炎症反应。

4 小 结

针刺百会、曲鬓穴可改善急性期ICH大鼠神经功能,减小血肿体积,促进血肿吸收,可能与针刺可促进CD36蛋白表达、抑制TLR4蛋白表达有关。

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Interventional Effect and Mechanism of Acupuncture at Baihui (GV 20) and Qubin (GB 7) Acupoints on Rats with Acute Cerebral Hemorrhage

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ABSTRACT Objective To investigate the interventional effect of acupuncture at Baihui (GV 20) and Qubin (GB 7) acupoints on the expression of recombinant cluster of differentiation 36 (CD36) and Toll-like receptor 4 (TLR4) in rats with acute cerebral hemorrhage (ICH), and to explore the mechanism of acupuncture treatment for ICH. **Methods** A total of 144 male Wistar rats were randomly divided into sham operation group, model group, acupuncture group and inhibitor group, with 36 cases in each group, and then each group was divided into three subgroups at the 1st, 3rd and 7th day, with 12 cases in each subgroup. The stereotaxic autologous blood injection method was used to establish a rat model of ICH. The model group only received ICH model preparation without any treatment. The sham operation group received various operations similar to the model group, but without blood injection. The inhibitor group received an intraperitoneal injection of the TLR4 inhibitor TAK242 at 6 hours after modeling, and the dose was 3 mg/kg, once daily for 5 days. At 12 hours after model establishment, all subgroups of the acupuncture group received penetrative needling at Baihui (GV 20) and Qubin (GB 7) acupoints. The needle was retained at a depth of 20 mm and twisted at a frequency of 100 r/min for three sessions of 2 min each at an interval of 5 min during the 30-min needle retention period. Acupuncture was given once a day and lasted for 1, 3, and 7 days in the three acupuncture subgroups, respectively. At the 1st, 3rd and 7th day after treatment, modified neurological severity score (mNSS) was used to assess neurological function. The volume of hematoma in brain tissue was detected. Western blot was used to detect the protein expression level of CD36 and TLR4 in brain tissue. Immunofluorescence method was used to detect the expression of CD36 and TLR4 in astrocytes. **Results** (1) mNSS score: compared with the sham operation group at the same time, mNSS score of the model group, the acupuncture group and the inhibitor group at 1, 3, 7 days after treatment increased significantly ($P<0.05$); compared with the model group at the same time, mNSS score of the acupuncture group and the inhibitor group at 1, 3, 7 days after treatment decreased significantly ($P<0.05$); compared with the acupuncture group at the same time, mNSS score in the inhibitor group at 1 day after treatment decreased significantly ($P<0.05$). (2) Hematoma volume: compared with the model group at the same time, hematoma volume in the acupuncture group and the inhibitor group at 3, 7 days after treatment decreased significantly, and hematoma volume in the inhibitor group at 1, 3, 7 days after treatment decreased significantly ($P<0.05$); compared with the acupuncture group at the same time, hematoma volume in the inhibitor group decreased significantly ($P<0.05$). (3) Protein expression level of CD36 and TLR4: compared with the sham operation group at the same time, the protein expression level of CD36 and TLR4 in the model group, the acupuncture group and the inhibitor group at 1, 3, 7 days after treatment increased significantly ($P<0.05$); compared with the model group at the same time, the expression level of CD36 protein in the acupuncture group and the inhibitor group at 1, 3, 7 days after treatment increased significantly ($P<0.05$), and the expression level of TLR4 protein in the acupuncture group and the inhibitor group at 3, 7 days after treatment decreased significantly ($P<0.05$); compared with the acupuncture group, the expression level of CD36 protein in the inhibitor group at 1, 3, 7 days after treatment increased significantly, and the expression level of TLR4 protein decreased significantly ($P<0.05$). (4) Expression level of CD36 and TLR4 in GFAP: there was a small amount of CD36 and TLR4 in the brain tissue of rats in the sham operation group. Compared with the sham operation group at the same time, the expression of CD36 and TLR4 in GFAP in the model group at 1, 3, 7 days after treatment increased significantly ($P<0.05$); compared with the model group at the same time, the expression of CD36 in GFAP of the inhibitor group and the acupuncture group at 1, 3, 7 days after treatment increased significantly, while the expression of TLR4 in GFAP decreased significantly ($P<0.05$). **Conclusion** Acupuncture at Baihui (GV 20) and Qubin (GB 7) acupoints can improve neurological function of rats with acute ICH, alleviate hematoma volume, which may be related to promoting the expression of CD36 protein and inhibiting the expression of TLR4 protein.

KEY WORDS cerebral hemorrhage; acupuncture; Baihui (GV 20); Qubin (GB 7); neurological function; CD36; TLR4

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