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电针对大鼠心肺复苏后脑损伤的保护作用 及对海马炎性因子表达的影响*

刘永飞¹ 张莉² 白卫平¹ 李娟¹ 张贺铭¹ 高昌俊^{1Δ}

(1 空军军医大学唐都医院麻醉科 陕西 西安 710038; 2 空军军医大学西京医院麻醉科 陕西 西安 710032)

摘要 目的:探讨电针对大鼠心肺复苏后脑损伤及海马炎性因子表达的影响。**方法:**雄性 SD 大鼠随机分三组:假手术组(Sham)、对照组(Control)、电针组(EA)。大鼠窒息 8 min 后进行心肺复苏,EA 组于复苏同时在水沟、内关穴插入毫针并予以电针刺激,对照组仅在相同穴位插入毫针。计算大鼠复苏成功率,记录自主循环恢复时间,于复苏后 24 h 及 72 h 对大鼠进行神经功能缺损评分(NDS),水迷宫检测各组大鼠学习记忆能力,尼氏染色观察海马区神经元形态及存活数量,Western blot 检测海马区炎性因子表达。**结果:**与 Sham 组相比,对照组与 EA 组大鼠复苏后 24 h、72 h NDS 降低,学习记忆能力明显减低,两组海马 CA1 区细胞排列紊乱、神经元存活数量减少,IL-10 表达降低,IL-1 与 IL-6 表达升高($P<0.05$)。而与对照组相比,EA 组大鼠复苏成功率有所提高,但无统计学意义,自主循环恢复时间明显缩短($P<0.05$);复苏后 24 h、72 h NDS 评分提高($P<0.05$);水迷宫第六天逃避潜伏期缩短、空间探索能力显著增强($P<0.05$);海马 CA1 区细胞排列紊乱减轻,神经元存活数目增多;海马区炎性因子 IL-1、IL-6 表达降低,抗炎因子 IL-10 表达增多($P<0.05$)。**结论:**电针能够减轻大鼠心肺复苏后脑损伤,其保护作用可能与抑制炎性因子、促进抗炎因子表达有关。

关键词:电针;心肺复苏;心脏骤停;水沟穴;内关穴

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Protective Effect of Electroacupuncture on Brain Injury after Cardiopulmonary Resuscitation in Rats and Its Effect on Expression of Inflammatory Factors in Hippocampus*

LIU Yong-fei¹, ZHANG Li², BAI Wei-ping¹, LI Juan¹, ZHANG He-ming¹, GAO Chang-jun^{1Δ}

(1 Department of Anesthesiology, Tangdu Hospital, AirForce Military Medical University, Xi'an, Shanxi, 710038, China;

2 Department of Anesthesiology, Xijing Hospital, AirForce Military Medical University, Xi'an, Shaanxi, 710032, China)

ABSTRACT Objective: To investigate the effect of electroacupuncture (EA) on brain injury and expression of inflammatory factors in hippocampus after cardiopulmonary resuscitation in rats. **Methods:** Male SD rats were randomly divided into three groups: sham group, control group and EA group. Rats were asphyxiated for 8min before cardiopulmonary resuscitation (CPR). The EA group was inserted filiform needles in the shuigou and neiguan points and received electrical stimulation, while the control group was only inserted filiform needles at the same points. The survival rate of rat resuscitation and the recovery time of spontaneous circulation were recorded. Neurological deficit score (NDS) was measured at 24 h and 72 h after resuscitation. The spatial learning and memory ability of each group was tested by Morris water maze. Neuronal morphology and survival in hippocampus were observed by Nissl staining. The expression of inflammatory factors in hippocampus was detected by Western blot. **Results:** Compared with the sham group, the NDS of rats at 24 h and 72 h after resuscitation and the spatial learning and memory ability were significantly reduced in other two groups. In this two groups, hippocampal CA1 cells were disordered, meanwhile, the number of viable neurons, the expression of IL-10 were decreased, but IL-1 and IL-6 were increased when compared with sham group ($P<0.05$). Compared with the control group, the survival rate was improved without statistically significant, the recovery time of spontaneous circulation was shortened ($P<0.05$), the NDS was increased at 24 h and 72 h after resuscitation ($P<0.05$), the escape latency of 6th day of water maze was shortened and the space exploration ability was promoted in the EA group ($P<0.05$). The cell line in the hippocampal CA1 and the number of viable neurons in the EA group were significantly better than that of control group. IL-1 and IL-6 in the hippocampus of EA group were alleviated, while the expression of anti-inflammatory factor IL-10 was enhanced compared with the control group ($P<0.05$). **Conclusion:** Electroacupuncture can alleviate brain injury after cardiopulmonary resuscitation in rats, and its protective effect may be related with inhibiting inflammatory factors and promoting the expression of anti-inflammatory factors.

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作者简介:刘永飞(1992-), 硕士, 研究方向:心肺复苏后器官保护, 电话:15339046237, E-mail: 843117101@qq.com

Δ 通讯作者:高昌俊, 博士, 副教授, 研究方向:心肺复苏后器官保护, E-mail: gaocj74@163.com

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前言

心跳骤停是严重的危重症,虽然心肺复苏技术的普及和其它辅助措施应用提高了复苏成功率,但脑保护效果依然不令人满意^[1-3]。如何有效修复损伤的神经元,减轻复苏后神经系统并发症,提高心肺复苏后患者的存活率和生存质量,仍是亟待解决的临床医学难题。电针有着操作简单、方便可控、易于掌握、副作用小等优点。研究表明电针可以通过抑制 TLR4/NF- κ B, p38 MAPK 和 MyD88 信号通路,激活胆碱能抗炎通路等,调节炎症反应,从而减少大鼠脑梗后的梗死体积,改善脑缺血引起的神经功能缺损达到脑保护效应^[4-6]。有关电针对心肺复苏后脑保护的研究较少,本研究拟探讨电针刺激内关穴和水沟穴对大鼠心肺复苏后脑损伤的保护作用,并观察损伤后海马炎症因子表达的变化。

1 材料与方法

1.1 大鼠模型制备

模型参照文献^[7]中的方法建立。大鼠禁食 12 h,自由饮水,腹腔注射 1%戊巴比妥钠 40-50 mg·kg⁻¹ 进行麻醉,固定于操作台上。左侧腹股沟剪毛、酒精消毒,暴露股动脉鞘并分离股动脉及静脉,动脉接监护仪监测血压,静脉预留给药。自制气管导管经口插入气管,接动物呼吸机,自主呼吸 5 min 后夹闭气管导管,8 min 后进行机械通气(呼吸机参数:潮气量 6 mL·kg⁻¹,频率 80 次/分,呼吸比 1:1),静脉注射肾上腺素 0.02 mg·kg⁻¹ 和碳酸氢钠 1 mmol·kg⁻¹,同时三指法胸外按压(频率 160 次/分,幅度为胸廓前后径的 1/3)。心脏停搏定义为平均动脉压(MAP)<25 mmHg,当 MAP>50 mmHg 且有自主心跳并持续 10 min 为自主循环恢复,抢救 10 min 无效为复苏失败。自主呼吸恢复到正常水平后拔管,缝合左侧腹股沟切口、消毒,将大鼠放回笼中。

1.2 电针处理

穴位参照《华兴邦大鼠穴位图谱的研制》,使用毫针(0.35×13 mm)于大鼠左前肢内侧距腕关节 3 mm 左右尺桡骨缝间的内关穴上直刺 1 mm,唇裂鼻尖下 1 mm 正中处的水沟穴直刺 1 mm。电子针疗仪(华佗牌,SDZ-V)两极接于穴位构成回路,刺激频率 20 Hz、疏密波形、强度为 3,大鼠复苏同时予以刺激,持续 30 min,1 h 后再刺激 30 min。

1.3 动物分组

成年雄性 SD 大鼠,由空军军医大学动物实验中心提供。随机分成 3 组,分别为 Sham 组(10 只)、对照组(20 只)、EA 组(20 只)3 组。EA 组于复苏同时在水沟、内关穴插入毫针并予以电针刺激;对照组仅在相同穴位插入毫针不给予电刺激;Sham 组仅气管插管、左侧股动静脉穿刺。

1.4 复苏成功率及自主循环恢复时间

计算对照组和 EA 组大鼠复苏成功率,对复苏成功的大鼠记录其自主循环恢复时间,该时间指复苏开始至大鼠有自主心

跳且平均动脉压达到 50 mmHg 结束。

1.5 神经功能缺损评分

各组存活的大鼠随机选取 5 只,参照 Geocadin[®]神经功能缺损评分量表,于复苏后 24 h 及 72 h 进行评分,具体内容包括总体表现、脑干功能、运动评估、感觉评估、共济功能以及癫痫等方面评分,范围 80 分(完全正常)至 0 分(脑死亡)。

1.6 水迷宫的测试

参照 Morris^[10]水迷宫实验,采用 TM-vision 行为学实验系统(泰盟),水池分 4 个象限,将无色透明平台放入第四象限中间,使平台最高点低于水面 2 cm,加热棒加热水温至 26℃。复苏后的第四天,在平台所对象限将大鼠头朝池壁放入水中进行获得性训练,观察 60 s,记录大鼠找到平台时间即逃避潜伏期,若 60 s 内未找到平台,将动物引入平台并停放 10 s,移开动物擦干。每天 3 次,每次间隔时间 20 min,第五、六天同上,记录大鼠每天平均潜伏期。第七天将平台移出,进行探索性试验,记录动物滞留平台象限时间及穿越平台次数。

1.7 尼氏染色

水迷宫测试后的大鼠,麻醉后分别用生理盐水、4%多聚甲醛灌注后取脑。将大脑浸泡在 4%多聚甲醛 2 小时,随后依次经 10%、20%、30%蔗糖梯度脱水,于冰冻切片机上切片。脑片厚度 20 μ m,贴片后采用焦油紫染色 2 h,再依次分化、脱水、透明、封片。晾干后分别在 50 倍、400 倍显微镜下观察海马区域尼氏小体数量及神经元形态。

1.8 Western blot

每组存活 24 h 后的大鼠随机取 5 只,麻醉后断头,在冰上分离海马区,加入 RIPA 裂解液和 PMSF 组织匀浆,12000 r·min⁻¹,4℃离心 10 min 取上清,提总蛋白并用 BCA 法测定其浓度。按照凝胶试剂盒说明书配置凝胶。凝固后上样,上层胶 80v,下层胶 120v 电泳,完毕后 100v 转膜 50 min。将转好的 PVDF 膜放于 5% BSA 中封闭 1 h 后分别加入 IL-10、IL-6、IL-1、 β -actin 4℃过夜。洗膜后二抗孵育 1 h,PBST 洗三次。Chemicon MP 扫描系统发光显影并进行分析。

1.9 统计学分析

采用 SPSS13.0 统计软件进行数据分析,计量资料以均数 $\pm$ 标准差($\bar{x}\pm s$)表示,采用单因素方差分析进行差异性比较,两两比较采用 SNK 检验。大鼠复苏成功率采用 χ^2 检验。神经缺损评分以中位数和四分位间距表示,组间比较采用 Kruskal-Wallis 检验,两两比较采用 Mann-Whitney U 检验。 $P<0.05$ 认为差异有统计学意义。

2 结果

各组大鼠窒息前的体重、平均动脉压的差异无统计学意义(表 1);复苏成功数分别为对照组 10 (50%) 只,电针组 14 (70%) 只,EA 组复苏成功率高于对照组(表 1),但是没有统计学差异;与对照组相比,EA 组的循环恢复时间缩短($P<0.05$)(图 1);与 Sham 相比,各组 NDS 评分降低,而 EA 组 24 h 及 72 h NDS 评分均高于对照组($P<0.05$)(表 2)。

表 1 各组大鼠一般情况及复苏成功率($\bar{x} \pm s$)

Table 1 General conditions and recovery success rate ($\bar{x} \pm s$)

Groups	n	Weight(g)	MAP (mmHg)	Survival rate [n(%)]
Sham	10	302.00 $\pm$ 9.53	102.2 $\pm$ 7.0	--
Control	20	290.05 $\pm$ 20.42	101.8 $\pm$ 7.0	10(50)
EA	20	290.95 $\pm$ 24.15	104.0 $\pm$ 6.5	14(70)

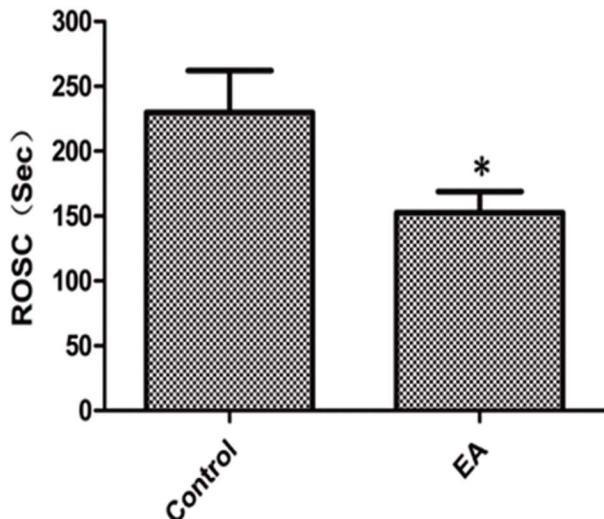


图 1 大鼠自主循环恢复时间比较

Fig.1 The recovery time of spontaneous circulation in rats

Note: * $P < 0.05$ compared with the Control.

表 2 神经功能缺损评分[分,M(IQR)]

Table 2 Neurological deficit scores at 24 h and 72 h after ROSC

Groups	n	24 h	72 h
Sham	5	80.00 (79.00,80.00) [#]	80.00 (80.00,80.00) [#]
Control	5	40.00 (36.50,52.00)	55.00 (49.00,63.50)
EA	5	55.00 (54.00,60.50)*	69.00 (65.00,75.00)*

Note: [#] $P < 0.05$ compared with the other groups, * $P < 0.05$ compared with the Control.

水迷宫训练显示,随着训练次数、天数的增多,各组大鼠逃避潜伏期均逐渐缩短。与对照组相比,EA 组大鼠复苏后的第六天逃避潜伏期明显缩短($P < 0.05$)(表 3),空间探索期间于平台所在象限滞留时间明显延长($P < 0.05$),穿过平台次数虽然多于对照组但无统计学差异(图 2)。

表 3 各组大鼠水迷宫训练期间逃避潜伏期($S, \bar{x} \pm s$)

Table 3 Latency time during water maze training (Sec)

Groups	n	4 d	5 d	6 d
Sham	5	41.00 $\pm$ 4.45	20.46 $\pm$ 1.24 [#]	13.07 $\pm$ 5.60 [#]
Control	5	57.66 $\pm$ 3.53	52.69 $\pm$ 4.47	37.81 $\pm$ 7.85
EA	5	56.15 $\pm$ 6.03	46.19 $\pm$ 11.35	25.60 $\pm$ 5.46*

Note: [#] $P < 0.05$ compared with the other groups, * $P < 0.05$ compared with the Control.

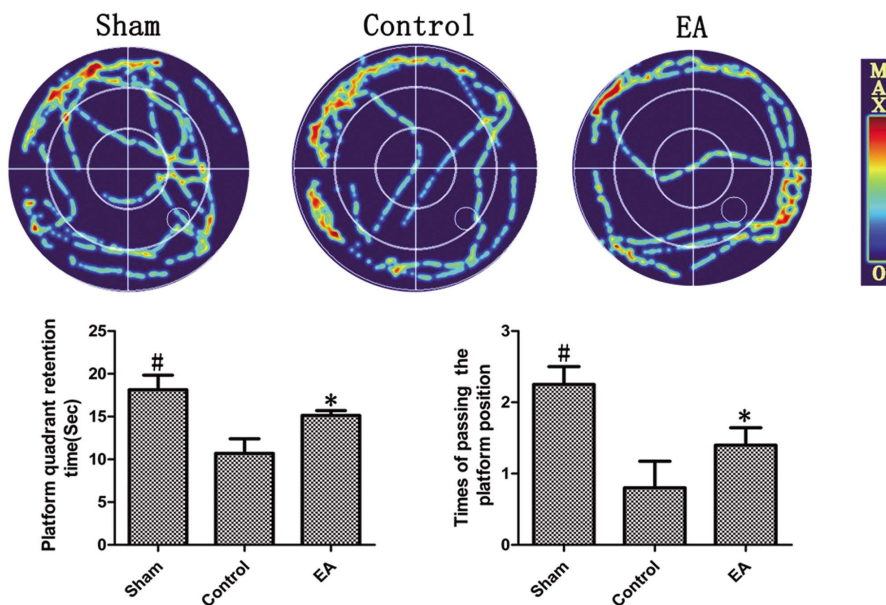


图 2 各组大鼠平台象限滞留时间及进入平台次数

Fig.2 Platform quadrant retention time and times of passing the platform position of rats in each group at 7th day

Note: [#] $P < 0.05$ compared with the other groups, * $P < 0.05$ compared with the Control.

与 Sham 组比较,对照组和 EA 组海马区细胞层数减少、排列紊乱,存活神经元数目及抗炎因子 IL-10 表达都降低,炎症因子 IL-1 和 IL-6 表达增多($P<0.05$);与对照组比较,EA 组海

马区细胞排列紊乱明显减轻,存活神经元数目及 IL-10 表达增多,IL-1 和 IL-6 表达降低($P<0.05$)(图 3、图 4)。

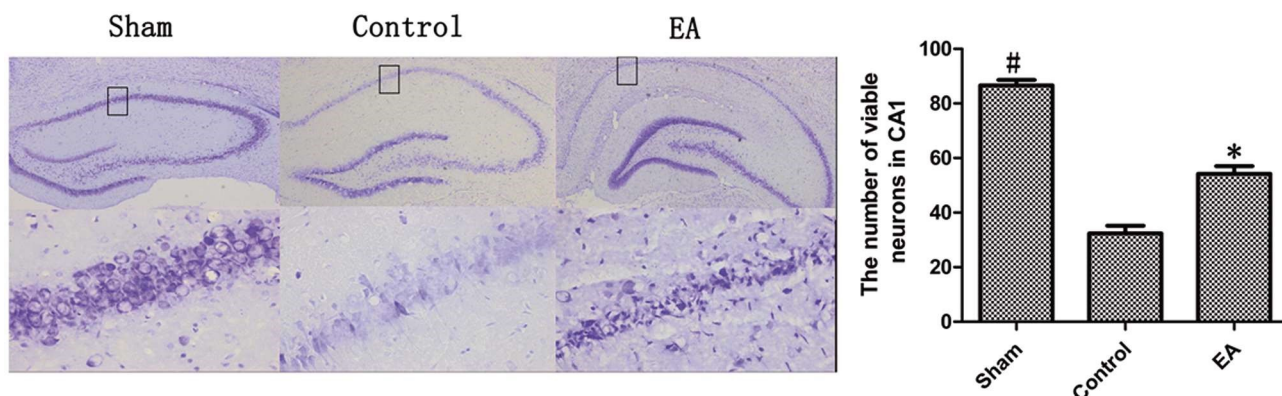


图 3 海马区尼氏染色及存活神经元的比较(50 倍, 400 倍)

Fig.3 Nissl staining of hippocampus in the 3 groups and the number of viable neurons of the CA1 areas (magnification $\times 50, \times 400$)

Note: # $P<0.05$ compared with the other groups, * $P<0.05$ compared with the Control.

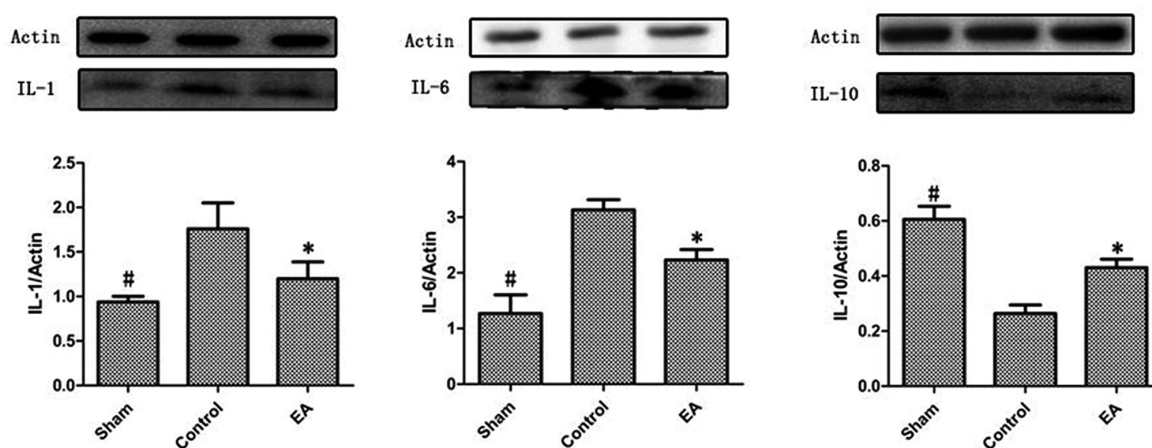


图 4 复苏后各组大鼠海马区炎症因子的表达

Fig.4 Expression of inflammatory factors after resuscitation in each group of rats

Note: # $P<0.05$ compared with the other groups, * $P<0.05$ compared with the Control.

3 讨论

电针刺激穴位作为传统医学与现代技术结合的医疗手段,广泛应用于临床及基础研究中。《灵枢经脉》中提到内关穴是手厥阴心包经的常用腧穴,《内经》认为:"心为人身之君主,不得受邪,若外邪侵心,则心包当先受病。"心包经可以调节心脏的功能,促进心脏功能恢复;水沟又名人中,上行人脑是督脉与手足阳明经的交汇之穴,起到醒神开窍的疗效。研究表明电针刺激水沟穴或内关穴能改善大鼠缺血再灌注引起的脑损伤^[11,12]。本实验中窒息性心跳骤停引起的全脑缺血损伤模型,模拟了麻醉过程因气道阻塞造成心脏停跳的意外情况,我们通过观察不同指标来探讨电针刺激水沟、内关穴对大鼠心肺复苏后脑损伤的影响。

临床证据表明,心跳骤停后,随着心肺复苏的时间延长,患者存活率就会下降^[13,14],而自主循环恢复后能够维持冠状动脉灌注压,促进心功能的恢复,进一步改善大脑缺血缺氧的状态^[15]。因此,尽快恢复自主循环是脑损伤预后良好的关键。NDS是从各个方面对大鼠神经功能的评估,能反映复苏后大鼠预后的情

况。本实验观察到电针联合刺激内关、水沟穴能够降低大鼠自主循环恢复时间,提高复苏后 24 h、72 h 神经功能缺损评分,说明电针减轻心肺复苏后大鼠脑损伤可能与改善心脏功能有关。

大脑缺血缺氧是心脏骤停造成神经损伤的主要原因。海马区对缺血缺氧的耐受性最差,最易受损的部位,影响着学习能力和记忆功能^[16]。水迷宫作为行为学检测方法之一,可以有效反映大鼠学习、记忆、空间搜索能力^[17]。本研究通过水迷宫测试观察到,电针刺激缩短心肺复苏后大鼠逃避潜伏期,提高学习、记忆空间探索能力,促进行为恢复。除此之外,海马区神经元坏死数量也直接反映了海马损伤程度,尼氏染色观察到电针刺激增加了心肺复苏后大鼠海马神经元存活数量,EA 组海马细胞排列紊乱较对照组明显减轻,这表明电针对心脏骤停后海马损伤有一定保护作用。

心跳停止引起脑缺血,短时间内脑组织即可发生炎症反应,自主循环恢复后,大脑缺血再灌注也会过度产生炎症因子,都是导致神经元损伤的重要原因^[18,20]。有学者观察到缺血后的炎症损伤比缺血本身对脑组织损伤更为严重,脑缺血损伤后采取抑制炎症反应的措施可以改善实验动物的预后^[19]。有研究表

明心肺复苏后一些炎症因子如 IL-1、IL-6 会明显增高, 采取保护措施(低温、药物)后这些炎症因子都有所降低, 起到相应的治疗作用^[21,22], 还有实验证明电针可以减少脑缺血大鼠海马炎症因子 IL-1、TNF- α 表达。我们课题组在之前的研究中^[23]也观察到, 窒息性心脏骤停大鼠模型中, 脑组织 IL-1 和 TNF- α 致炎因子含量升高。此次研究结果显示电针减少了脑组织中炎症因子 IL-1、IL-6 含量, 这可能与脑保护有一定关联。我们在实验中还观察到, EA 组海马组织抗炎因子 IL-10 的表达明显高于对照组, 这与 Grace 等^[24]的研究结果一致。因此, 我们推测电针减轻心肺复苏后大鼠脑损伤, 可能与其调节促炎因子 IL-1、IL-6 和抗炎因子 IL-10 的平衡, 降低机体炎症反应强度有关。

综上所述, 电针联合刺激水沟、内关穴能够缩短窒息性心脏骤停大鼠的自主循环恢复时间, 调节脑组织中致炎、抗炎因子含量, 减少神经元损伤, 提高大鼠学习记忆能力, 改善大鼠预后。不过, 本实验还有些不足之处, 比如没有观察电针刺激其它穴位是否也有类似效应, 另外, 电针对炎症因子的内在调控机制并未进行深入探讨。因此, 电针对心肺复苏脑损伤保护作用的研究任重而道远。

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