

· 基础研究 ·

低频磁疗对脱髓鞘模型小鼠髓鞘及炎症反应的影响

缪晓影 郑碧娥 苏玲玲 何任红 范建中 尹瑞雪

南方医科大学南方医院康复医学科, 广州 510515

通信作者: 尹瑞雪, Email: yinruixueshen@126.com

【摘要】 目的 探讨低频磁疗对双环己酮草酰二胺(CPZ)诱导的脱髓鞘模型小鼠胼胝体区髓鞘及炎症反应的影响。**方法** 将36只6~8周龄雄性C57BL/6J小鼠按随机数字表法分为对照组、CPZ组和磁疗组。对照组给予常规饲料饲养6周, CPZ组和磁疗组均用含0.3% CPZ混合饲料饲养6周, 诱导脱髓鞘模型小鼠。磁疗组于开始喂食CPZ行磁疗干预6周, 磁场强度为10 mT, 频率为50 Hz。每隔7 d观察小鼠体质量。于第6周末行高架十字迷宫实验观察小鼠焦虑状态, 采用卢卡斯快蓝(LFB)染色和髓鞘碱性蛋白(MBP)免疫组化技术观察胼胝体区髓鞘, 采用酶联免疫吸附(ELISA)法检测胼胝体区肿瘤坏死因子- α (TNF- α)和白细胞介素-1 β (IL-1 β)含量。**结果** 干预6周后, 磁疗组的体质量较CPZ组明显改善($P<0.05$)。CPZ组的进入开臂次数[(7.75 $\pm$ 2.05)次]、开臂内滞留时间百分比[(8.13 $\pm$ 2.34)%]和进入开臂次数百分比[(20.46 $\pm$ 3.04)%]均较对照组[(12.50 $\pm$ 3.70)次、(25.75 $\pm$ 2.20)%和(43.49 $\pm$ 2.98)%]显著减少($P<0.01$); 而与CPZ组比较, 磁疗组的进入开臂次数[(10.00 $\pm$ 2.05)次]和进入开臂次数百分比[(24.23 $\pm$ 5.63)%]均明显增多($P<0.05$), 且开臂内滞留时间百分比[(15.81 $\pm$ 6.07)%]显著升高($P<0.01$)。LFB染色显示, CPZ组胼胝体区髓鞘脱失明显, 与CPZ组相比, 磁疗组胼胝体区髓鞘脱失明显改善, MBP免疫组化染色进一步证实了这一结果, CPZ组MBP表达量较对照组明显减少($P<0.01$); 而与CPZ组相比, 磁疗组MBP表达量明显增多($P<0.01$)。与对照组相比, CPZ组的TNF- α 和IL-1 β 的蛋白水平明显升高($P<0.01$); 与CPZ组相比, 磁疗组胼胝体区TNF- α 和IL-1 β 的蛋白水平均显著降低($P<0.01$)。**结论** 低频磁疗可以改善小鼠体重和焦虑状态, 其机制可能与改善胼胝体区髓鞘脱失及抑制炎症反应有关。

【关键词】 低频磁疗; 双环己酮草酰二胺; 脱髓鞘; 焦虑; 炎症反应

基金项目: 广东省自然科学基金项目(2016A030313523); 南方医科大学南方医院院长基金项目(2019B024)

DOI: 10.3760/cma.j.issn.0254-1424.2021.10.001

Effects of low frequency magnetic stimulation on myelin and inflammation in demyelinated mice

Miao Xiaoying, Zheng Bie, Su Lingling, He Renhong, Fan Jianzhong, Yin Ruixue

Department of Rehabilitation Medicine, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China

Corresponding author: Yin Ruixue, Email: yinruixueshen@126.com

【Abstract】 Objective To explore the effect of low frequency magnetic stimulation on myelin and inflammation in the callosum of demyelinated mice. **Methods** Thirty-six 6 to 8-week-old male C57BL/6J mice were randomly divided into a control group, a cuprizone (CPZ) group and a magnetic therapy group. The CPZ group and the magnetic therapy group had demyelination induced by feeding a mixed diet containing 0.3% CPZ for 6 weeks, while the control group was given conventional food. The magnetic therapy group was given 50Hz 10mT magnetic stimulation during the 6 weeks for 20min daily, 5 days a week. The body mass of each mouse was observed every 7 days. At the end of the 6th week elevated cross maze experiments were conducted to observe any anxiety state. The myelin sheath in the corpus callosum was observed using Luxol fast blue staining and myelin basic protein (MBP) immunohistochemistry. Tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) in the corpus callosum were detected using enzyme-linked immunosorbent assays. **Results** After the 6 weeks of treatment, the average body mass of the mice in the magnetic therapy group had improved significantly compared with the CPZ group. The CPZ group's times in the elevated cross maze experiments were significantly shorter than those of the control group and also shorter than those of the magnetic therapy group. The Luxol staining showed significant myelin loss in the corpus callosum of the CPZ group, but compared with the CPZ group the average loss of myelin in the magnetic

therapy group was significantly less. This was further confirmed by the MBP immunohistochemistry. Compared with the control group, the average expression of MBP in the CPZ group was significantly reduced, while in the magnetic therapy group it was significantly increased. Compared with the control group, the average TNF- α and IL-1 β levels in the corpus callosum of the CPZ group increased significantly, but compared with the CPZ group the average levels in the magnetic therapy group had decreased significantly. **Conclusions** Low frequency magnetic stimulation improves the body weight and anxiety state of mice. That is probably related to less myelin loss and inhibited inflammatory response in the corpus callosum.

[Key words] Low frequency magnetic therapy; Cuprizone; Demyelination; Anxiety; Inflammatory response

Funding: Natural Science Foundation of Guangdong Province (2016A030313523); The Presidential Funding from Nanfang Hospital of Southern Medical University (2019B024)

DOI:10.3760/cma.j.issn.0254-1424.2021.10.001

多发性硬化是一种累及中枢神经系统的慢性疾病,主要表现为炎症、广泛的原发性脱髓鞘和进行性神经退行性变^[1]。多发性硬化的病因和发病机制至今尚无定论,可能与病毒感染、免疫、遗传、环境等多种因素有关。目前认为,自身免疫反应是导致多发性硬化的主要机制,尤其是免疫炎症反应。自身免疫反应的靶点是少突胶质细胞髓鞘,外周血 T 淋巴细胞激活进入中枢神经系统后,进一步被特异性抗原激活,分泌肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)、白细胞介素-1 β (interleukin-1 β , IL-1 β) 等炎症性细胞因子及其它毒性损伤因子,如氧自由基 (oxygen radical, ROS)、一氧化氮 (nitric oxide, NO)、活性氮自由基 (reactive nitrogen species, RNS) 等,直接或通过巨噬细胞攻击以髓鞘成分为主的靶抗原,从而造成髓鞘组织破坏,导致神经症状的出现^[2-3]。

磁疗是临床常用的物理治疗方法,已被成功地用于多种临床条件下的辅助治疗。大量研究证实,磁场具有良好的抗炎作用^[4],可以增强细胞活性^[5],促进神经再生,改善神经功能^[6]。虽然有研究发现磁疗可以有效改善多发性硬化患者症状^[7-8],但其在改善脱髓鞘病变中的具体作用机制尚不清楚。本研究通过双环己酮草酰二胺 (cuprizone, CPZ) 诱导脱髓鞘小鼠模型,观察低频磁疗对小鼠胼胝体髓鞘及炎症反应的影响。

材料与方法

一、实验动物及分组

选取 36 只健康 6~8 周龄雄性 C57BL/6J 小鼠,清洁级,体质量 (20 $\pm$ 1) g,均由南方医科大学南方医院实验动物中心提供 (许可证号 SCXK 粤 2016-0041); 采用随机数字表法将小鼠分为对照组、CPZ 组及和磁疗组,每组 12 只。本实验经南方医科大学南方医院实验动物伦理委员会批准,所有动物实验操作均遵循伦理委

员会的相关规定。对照组用常规饲料饲养,CPZ 组和磁疗组用含 0.3% CPZ 混合饲料连续饲养 6 周。所有小鼠自由摄食饮水,环境温度 22~24 $^{\circ}\text{C}$,保证昼夜节律,避免强光和噪声刺激。每隔 7 d 测量 1 次小鼠体质量,监测小鼠体质量变化。

二、主要试剂及仪器

CPZ (美国 Sigma 公司); 卢卡斯快蓝 (Luxol fast blue, LFB) 髓鞘染色试剂盒 (货号 G1030, 武汉谷歌生物); 一抗 (1 : 600); 抗髓鞘碱性蛋白 (myelin basic protein, MBP) 抗体 (货号 GB12226, 武汉赛维尔生物科技有限公司); 二抗 (1 : 200); HRP 标记山羊抗小鼠 (货号 GB23301, 武汉赛维尔生物科技有限公司)。TNF- α 小鼠酶联免疫吸附 (enzyme-linked immunosorbent assays, ELISA) 试剂盒 (美国赛默飞世尔科技公司); IL-1 β 小鼠 ELISA 试剂盒 (美国赛默飞世尔科技公司); 低频磁疗仪 (型号 DC-4, 汕头市医用设备厂有限公司)。

三、磁疗干预方法

磁疗组小鼠在开始喂食 CPZ 第 1 天即进行磁疗干预,将小鼠置于固定器中,露出头部,异名磁极对置于头部,两磁极之间距离 15 cm,磁场强度为 10 mT,频率为 50 Hz, 20 min/次, 1 次/d, 5 次/周,共 6 周。CPZ 组小鼠同样被固定,过程与磁疗组一致,但不进行磁疗干预。

四、高架十字迷宫实验

于第 6 周末,采用高架十字迷宫实验评估小鼠的焦虑状态。提前 3 h 将小鼠置于行为学实验室适应环境,减轻小鼠紧张情绪。每次实验前均用 75% 乙醇擦净迷宫,待乙醇挥发干净再进行实验。实验开始时将小鼠置于高架十字迷宫的中间区域,头朝向开臂。让小鼠在迷宫中自由活动,实验过程应用监测分析系统 (上海欣软信息科技有限公司) 跟踪小鼠在高架十字迷宫中 5 min 的活动轨迹。用软件分析录像,计算每只小鼠的进入开臂次数、开臂内滞留时间百分比和进入开

臂次数百分比。

五、取材处理

完成行为学测试后,每组选取 6 只小鼠,用 0.5% 戊巴比妥钠腹腔注射麻醉小鼠,打开胸腔暴露心脏,剪开右心耳作为血液流出口,经左心室快速灌注生理盐水,至小鼠眼睛、四肢、尾巴出现苍白,迅速断头分离脑组织。将完整的脑组织立即放于 4% 多聚甲醛固定,常规石蜡包埋,取胼胝体的部位做连续矢状位切片,用于 LFB 染色及 MBP 免疫组化染色;再将剩余小鼠,以同样操作取出脑组织并分离胼胝体,放于 $-80\text{ }^{\circ}\text{C}$ 冷存。

六、LFB 染色

LFB 染色步骤:常规石蜡切片脱蜡至水,将切片放于 0.1% LFB 染液 $60\text{ }^{\circ}\text{C}$ 孵育过夜,蒸馏水冲洗;分化液中分色 15 s,蒸馏水冲洗,0.1%伊红复染 1 min,蒸馏水冲洗;烘箱吹干,二甲苯透明数分钟,中性树胶封片;光学显微镜下观察。

染色结果:神经髓鞘呈蓝色,其它成分呈红色。

七、MBP 免疫组化染色

免疫组化染色步骤:石蜡切片脱蜡至水,乙二胺四乙酸(ethylene diamine tetraacetic acid, EDTA) ($\text{pH}9.0$) 抗原修复,磷酸盐缓冲液(phosphate buffered solution, PBS) ($\text{pH}7.4$) 洗涤;3%双氧水室温避光孵育 25 min, PBS 洗涤;正常羊血清室温封闭;加一抗 $4\text{ }^{\circ}\text{C}$ 孵育过夜, PBS 洗涤;加二抗室温孵育 50 min, PBS 洗涤;二氨基联苯胺(diaminobenzidine, DAB)显色,苏木素复染;酒精脱水,二甲苯透明,中性树胶封片;显微镜镜检,图像采集。将 MBP 免疫组化染色结果用 Image Pro Plus 6.0 图像分析软件测定积分光密度(integral optical density, IOD)值和阳性面积(area),计算出各组切片平均光密度值(mean density = IOD/area),即 MBP 的含量。

八、TNF- α 及 IL-1 β 蛋白水平测定

ELISA 法检测胼胝体区 TNF- α 、IL-1 β 蛋白水平:取适量胼胝体组织标本,研磨匀浆,收集上清液,取适量上清液加入已包被抗体的酶标板孔内,室温孵育 2 h;采用试剂盒自带的洗涤液洗涤,各孔中加稀释好的生物素化抗体工作液室温孵育 1 h;洗涤,各孔中加稀释好的酶结合物工作液室温避光孵育 30 min;洗涤后进行四甲基联苯胺(tetramethylbenzidine, TMB)显色,用酶标仪在 450 nm 处测各孔吸光度值。根据标准品的浓度和吸光度值做标准曲线,然后根据标准曲线方程计算出样本浓度。

九、统计学方法

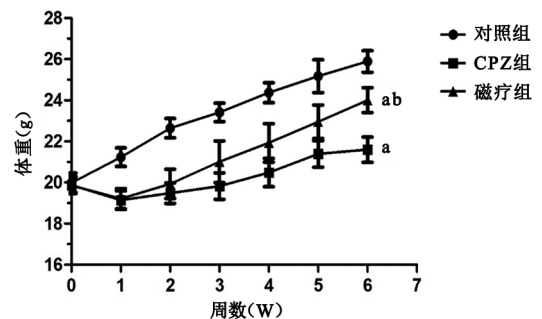
所有统计分析均使用 SPSS 23.0 统计软件包完成,计量资料以($\bar{x}\pm s$)表示,各组间均数比较采用单因

素方差分析,多组间两两均数比较采用 LSD 检验, $P<0.05$ 认为差异有统计学意义。

结 果

一、各组小鼠体质量的变化

体质量数据显示,对照组小鼠平均体质量呈现随时间稳定上升的趋势,而 CPZ 组和磁疗组小鼠平均体质量在第 1 周均出现迅速下降,第 2 周开始缓慢上升;在第 2~6 周,与 CPZ 组比较,磁疗组小鼠体质量上升的更明显;至第 6 周末,3 组小鼠两两间体质量比较,差异均有统计学意义($P<0.05$)。如图 1 所示。说明磁疗可以改善 CPZ 引起的体重降低。



注:与对照组比较,^a $P<0.05$;与 CPZ 组比较,^b $P<0.05$

图 1 各组小鼠体质量的变化

二、各组小鼠的焦虑状态

给予 CPZ 小鼠连续 6 周磁疗刺激后,高架十字迷宫实验结果显示,与对照组比较,CPZ 组小鼠的进入开臂次数、开臂内滞留时间百分比及进入开臂次数百分比均显著减少($P<0.01$),提示 CPZ 组小鼠出现焦虑样行为。与 CPZ 组比较,磁疗组小鼠的开臂内滞留时间百分比明显升高($P<0.01$),且进入开臂次数及进入开臂次数百分比亦显著增多($P<0.05$),详见表 1。提示低频磁疗能显著改善小鼠的焦虑样行为。

表 1 各组小鼠高架十字迷宫实验行为学指标比较($\bar{x}\pm s$)

组别	只数	进入开臂次数 (次)	开臂内滞留时间 百分比(%)	进入开臂次数 百分比(%)
对照组	12	12.50 $\pm$ 3.70	25.75 $\pm$ 2.20	43.49 $\pm$ 2.98
CPZ 组	12	7.75 $\pm$ 2.05 ^a	8.13 $\pm$ 2.34 ^a	20.46 $\pm$ 3.04 ^a
磁疗组	12	10.00 $\pm$ 2.05 ^{bd}	15.81 $\pm$ 6.07 ^{ac}	24.23 $\pm$ 5.63 ^{ad}

注:与对照组比较,^a $P<0.01$,^b $P<0.05$;与 CPZ 组比较,^c $P<0.01$,^d $P<0.05$

三、LFB 染色分析

LFB 染色结果如图 2 所示。对照组小鼠胼胝体区髓鞘染色较深且结构致密,排列整齐;CPZ 组染色较浅且颜色不均匀,髓鞘排列紊乱,空泡形成增多,提示髓鞘明显脱失;与 CPZ 组比较,磁疗组胼胝体区髓鞘染色较深,髓鞘轻度紊乱,较少空泡形成,说明经 6 周低频磁疗刺激后,小鼠胼胝体的脱髓鞘现

象明显改善。

四、MBP 免疫组化染色分析

MBP 免疫组化染色结果如图 3 所示。对照组胼胝体区域髓鞘结构致密,分布较均匀,平均光密度值 (IOD/area) 为 (25301.00 ± 2374.07) ; 而 CPZ 组髓鞘减少,结构紊乱,染色不均匀,平均光密度值为 (11900.67 ± 3340.02) ,与对照组比较,差异有统计学意义 ($P < 0.01$); 磁疗组胼胝体区髓鞘染色较深,结构改善,平均光密度值为 (18391.67 ± 4855.69) ,显著高于 CPZ 组 ($P < 0.01$),但仍低于对照组 ($P < 0.01$)。

五、各组小鼠胼胝体区 TNF- α 和 IL-1 β 蛋白水平

经 6 周干预后,与对照组比较,CPZ 组和磁疗组小鼠胼胝体组织中炎症因子 TNF- α 和 IL-1 β 的表达均明显升高 ($P < 0.01$); 而磁疗组胼胝体组织中 TNF- α 和 IL-1 β 的表达明显低于 CPZ 组,且组间差异有统计学意义 ($P < 0.01$),详见表 2。这说明低频磁疗刺激能抑制炎症因子的表达。

表 2 各组小鼠胼胝体 TNF- α 和 IL-1 β 水平比较
(pg/ml, $\bar{x} \pm s$)

组别	只数	TNF- α	IL-1 β
对照组	6	6.74 ± 1.92	8.00 ± 1.28
CPZ 组	6	24.21 ± 3.31^a	25.81 ± 1.55^a
磁疗组	6	14.87 ± 7.69^{ab}	13.34 ± 1.63^{ab}

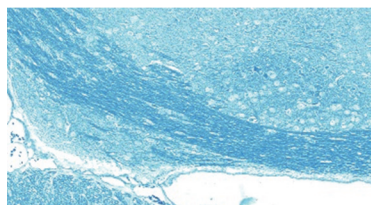
注:与对照组比较, $^a P < 0.01$; 与 CPZ 组比较, $^b P < 0.01$

讨 论

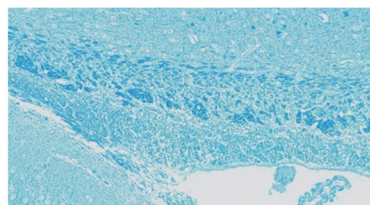
磁疗作为一种外加的物理刺激,产生的生物学效应较为复杂,许多实验证明适宜的磁场刺激可以预防炎症和氧化应激,减轻或修复神经系统损伤,磁场在多

种疾病中(包括阿尔兹海默病^[9]、帕金森病^[4]、缺血性卒中^[10]和疼痛^[5])均有保护作用。低频电磁场是指频率为 1~100 Hz,磁场强度 < 10 mT 的低频低强度磁场^[11]。国内有学者采用低频脉冲电磁场刺激脊髓损伤模型,发现磁疗可以降低炎症因子的表达,包括 IL-1 β 、TNF- α 及核因子- κ B (nuclear factor- κ B, NF- κ B)^[12]。亦有研究发现,低频率低强度的脉冲电磁场可以抑制炎症因子 IL-1 β 、TNF- α 的表达,起到缓解疼痛的作用^[13]。

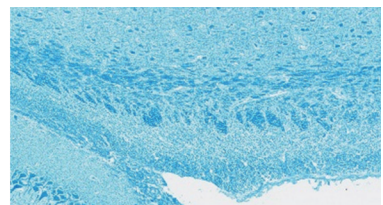
CPZ 作为一种铜离子螯合剂,在不直接影响其它胶质细胞的情况下,特异性地损害少突胶质细胞,并在 C57BL/6 小鼠大脑中诱导持续的脱髓鞘^[14]。因此,该化合物被普遍认为是动物模型中脱髓鞘和髓鞘再生的诱导剂^[15]。既往的研究表明,给小鼠饲喂 0.2%~0.3% 的 CPZ,第 2 周开始出现胼胝体髓鞘脱失,且脱髓鞘逐渐继续,并在第 5 周或第 6 周完成^[16-17]。本研究结果显示,CPZ 组小鼠平均体质量显著低于对照组,说明 CPZ 引起小鼠体质量降低;与对照组比较,CPZ 组小鼠 LFB 染色可见胼胝体髓鞘染色浅且排列紊乱,且 MBP 免疫组化显示 MBP 的含量下降;通过连续 6 周给予 0.3% CPZ 诱导小鼠胼胝体产生严重的脱髓鞘,成功建立脱髓鞘小鼠模型;磁疗组小鼠经磁疗干预 6 周后,与 CPZ 组比较,体质量上升更明显,表明磁疗可以改善 CPZ 引起的体重减轻;且磁疗组小鼠 LFB 染色显示脱髓鞘现象改善,MBP 含量表达升高,表明低频磁疗刺激可以有效减轻 CPZ 诱导的脱髓鞘。Li 等^[18]研究亦报道,磁疗刺激可以缓解髓鞘脱失,并促进髓鞘再形成,从而促进神经功能的恢复。



对照组

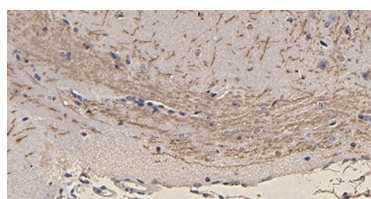


CPZ 组

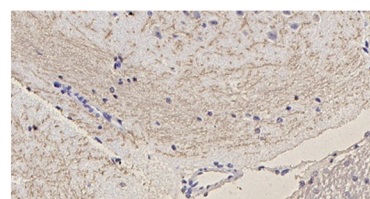


磁疗组

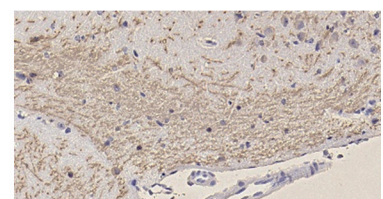
图 2 各组小鼠胼胝体 LFB 染色($\times 100$)



对照组



CPZ 组



磁疗组

图 3 各组小鼠胼胝体 MBP 免疫组化染色($\times 100$)

有研究表明,炎症参与了中枢神经系统的脱髓鞘,而多发性硬化的重要临床表现为焦虑状态,这种焦虑状态可以随脱髓鞘程度的减轻而得到缓解^[19-20]。高架十字迷宫实验是评估啮齿类动物焦虑状态的常用方法之一,进入开臂次数及开臂内滞留时间与小鼠的焦虑情绪成负相关,进入开臂次数越少,滞留时间越短,说明小鼠的焦虑情绪越严重。本研究行为学实验数据显示,CPZ 诱导的脱髓鞘小鼠进入开臂次数及滞留时间较对照组明显减少,表明 CPZ 可导致小鼠焦虑程度增加;而低频磁疗干预 6 周后,小鼠进入开臂次数、开臂内滞留时间百分比和进入开臂次数百分比明显增多,结果表明小鼠的焦虑状态明显缓解。结合低频磁疗改善髓鞘脱失的实验结果,说明磁疗改善小鼠焦虑状态可能与其改善 CPZ 诱导的脱髓鞘有关。

越来越多的证据表明,炎症反应在介导中枢神经系统的脱髓鞘中起着关键作用^[21]。研究报道,中枢神经系统炎症损伤是通过多种神经病理机制激活,IL-1 家族引发的炎症反应发挥神经毒性作用,激活渗入中枢神经系统的淋巴细胞,导致神经元和胶质细胞损伤^[22-23]。本研究结果表明,与 CPZ 组比较,磁疗组小鼠腓肠肌炎性因子 TNF- α 、IL-1 β 的表达显著降低,说明磁疗刺激能抑制炎症因子的表达,降低炎症损伤,减轻髓鞘脱失。研究发现,炎症参与脱髓鞘过程,而抑制炎症反应能有效减轻髓鞘脱失^[16, 24],这与本研究结果一致。

综上所述,低频磁疗可以改善 CPZ 诱导的髓鞘脱失,并有效缓解小鼠焦虑状态,抑制炎症因子 TNF- α 和 IL-1 β 的表达,磁疗对焦虑状态的改善可能与抑制髓鞘炎症反应及由此产生的神经保护作用有关。而低频磁疗引起炎症因子水平变化的具体分子机制仍有待于进一步深入研究。

参 考 文 献

- [1] Haider L, Zrzavy T, Hametner S, et al. The topography of demyelination and neurodegeneration in the multiple sclerosis brain[J]. Brain, 2016, 139:807-815. DOI:10.1093/brain/awv398.
- [2] Yadav SK, Mindur JE, Ito K, et al. Advances in the immunopathogenesis of multiple sclerosis[J]. Curr Opin Neurol, 2015, 28(3):206-219. DOI:10.1097/WCO.0000000000000205.
- [3] Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis[J]. Nat Rev Immunol, 2015, 15(9):545-558. DOI:10.1038/nri3871.
- [4] Bragin DE, Statom GL, Hagberg S, et al. Increases in microvascular perfusion and tissue oxygenation via pulsed electromagnetic fields in the healthy rat brain[J]. J Neurosurg, 2015, 122(5):1239-1247. DOI:10.3171/2014.8.JNS132083.
- [5] Elshawi AM, Hamada HA, Mosaad D, et al. Effect of pulsed electromagnetic field on nonspecific low back pain patients: a randomized

- controlled trial[J]. Braz J Phys Ther, 2019, 23(3):244-249. DOI:10.1016/j.bjpt.2018.08.004.
- [6] Pena-Philippides JC, Yang Y, Bragina O, et al. Effect of pulsed electromagnetic field (PEMF) on infarct size and inflammation after cerebral ischemia in mice[J]. Transl Stroke Res, 2014, 5(4):491-500. DOI:10.1007/s12975-014-0334-1.
- [7] Haase R, Piatkowski J, Ziemssen T. Long-term effects of Bio-Electromagnetic-Energy Regulation therapy on fatigue in patients with multiple sclerosis[J]. Altern Ther Health Med, 2011, 17(6):22-28.
- [8] Afshari D, Moradian N, Khalili M, et al. Evaluation of pulsing magnetic field effects on paresthesia in multiple sclerosis patients, a randomized, double-blind, parallel-group clinical trial[J]. Clin Neurol Neurosurg, 2016, 149:171-174. DOI:10.1016/j.clineuro.2016.08.015.
- [9] Capelli E, Torrisi F, Venturini L, et al. Low-frequency pulsed electromagnetic field is able to modulate miRNAs in an experimental cell model of Alzheimer's disease[J]. J Healthc Eng, 2017, 2017:2530270. DOI:10.1155/2017/2530270.
- [10] Urnukshaikhan E, Mishig-Ochir T, Kim S, et al. Neuroprotective effect of low frequency-pulsed electromagnetic fields in ischemic stroke[J]. Appl Biochem and Biotechnol, 2017, 181(4):1360-1371. DOI:10.1007/s12010-016-2289-z.
- [11] 魏清川, 李懿, 何成奇, 等. 低频脉冲电磁场治疗神经系统疾病的研究进展[J]. 循证医学, 2017, 17(6):373-376. DOI:10.12019/j.issn.1671-5144.2017.06.013.
- [12] Wang C, Liu Y, Wang Y, et al. Low-frequency pulsed electromagnetic field promotes functional recovery, reduces inflammation and oxidative stress, and enhances HSP70 expression following spinal cord injury[J]. Mol Med Rep, 2019, 19(3):1687-1693. DOI:10.3892/mmr.2019.9820.
- [13] 刘伟军, 王威, 黎清波, 等. 脉冲电磁场对人退变髓核细胞 A₂(2A) 腺苷受体的影响[J]. 中华物理医学与康复杂志, 2019, 41(11):818-822. DOI:10.3760/cma.j.issn.0254-1424.2019.11.004.
- [14] 穆建坤, 刘宏亮, 姚忠祥, 等. 大麻素 WIN55, 212-2 对双环己酮草酰二胺诱导的脱髓鞘模型髓鞘修复作用的研究[J]. 第三军医大学学报, 2013, 35(11):1129-1132. DOI:10.16016/j.1000-5404.2013.11.005.
- [15] Van der Star BJ, Vogel DY, Kipp M, et al. In vitro and in vivo models of multiple sclerosis[J]. CNS Neurol Disord Drug Targets, 2012, 11(5):570-588. DOI:10.2174/187152712801661284.
- [16] Yu H, Wu M, Lu G, et al. Prednisone alleviates demyelination through regulation of the NLRP3 inflammasome in a C57BL/6 mouse model of cuprizone-induced demyelination[J]. Brain Res, 2018, 1678:75-84. DOI:10.1016/j.brainres.2017.09.034.
- [17] Gudi V, Ginge S, Skripuletz T, et al. Glial response during cuprizone-induced de- and remyelination in the CNS: lessons learned[J]. Front Cell Neurosci, 2014, 8:37. DOI:10.3389/fncel.2014.00073.
- [18] Li Z, Yao F, Cheng L, et al. Low frequency pulsed electromagnetic field promotes the recovery of neurological function after spinal cord injury in rats[J]. J Orthop Res, 2019, 37(2):449-456. DOI:10.1002/jor.24172.
- [19] Mohamed A, Al-Kafaji G, Almahroos A, et al. Effects of enhanced environment and induced depression on cuprizone mouse model of demyelination[J]. Exp Ther Med, 2019, 18(1):566-572. DOI:10.3892/etm.2019.7654.

- [20] Silva BA, Leal MC, Farias MI, et al. Environmental enrichment improves cognitive symptoms and pathological features in a focal model of cortical damage of multiple sclerosis [J]. *Brain Res*, 2020, 1727: 146520. DOI:10.1016/j.brainres.2019.146520.
- [21] Shaw PJ, Lukens JR, Burns S, et al. Cutting edge: critical role for PYCARD/ASC in the development of experimental autoimmune encephalomyelitis [J]. *J Immunol*, 2010, 184(9): 4610-4614. DOI:10.4049/jimmunol.1000217.
- [22] Lee PR, Johnson TP, Gnanapavan S, et al. Protease-activated receptor-1 activation by granzyme B causes neurotoxicity that is augmented by interleukin-1 β [J]. *J Neuroinflammation*, 2017, 14(1): 131. DOI: 10.1186/s12974-017-0901-y.
- [23] Thornton P, Pinteaux E, Gibson RM, et al. Interleukin-1-induced neurotoxicity is mediated by glia and requires caspase activation and free radical release [J]. *J Neurochem*, 2006, 98(1): 258-266. DOI: 10.1111/j.1471-4159.2006.03872.x.
- [24] Liu M, Liu X, Wang L, et al. TRPV4 inhibition improved myelination and reduced glia reactivity and inflammation in a cuprizone-induced mouse model of demyelination [J]. *Front Cell Neurosci*, 2018, 12:392. DOI:10.3389/fncel.2018.00392.

(修回日期:2021-08-02)

(本文编辑:汪 玲)

· 外刊文献题录 ·

再生康复医学近期文献题录(一)

- [1] Yang B, Zhang F, Cheng F, et al. Strategies and prospects of effective neural circuits reconstruction after spinal cord injury [J]. *Cell Death Dis*, 2020, 11(6): 439.
- [2] Chalfouh C, Guillou C, Hardouin J, et al. The regenerative effect of trans-spinal magnetic stimulation after spinal cord injury: mechanisms and pathways underlying the effect [J]. *Neurotherapeutics*, 2020, 17(4): 2069-2088.
- [3] Zhao C, Xing Z, Zhang C, et al. Nanopharmaceutical-based regenerative medicine: a promising therapeutic strategy for spinal cord injury [J]. *J Mater Chem B*, 2021, 9(10): 2367-2383.
- [4] Wang J, Kong X, Li Q, et al. The spatial arrangement of cells in a 3D-printed biomimetic spinal cord promotes directional differentiation and repairs the motor function after spinal cord injury [J]. *Biofabrication*, 2021, 13(4).
- [5] Matthews J, Surey S, Grover LM, et al. Thermosensitive collagen/fibrinogen gels loaded with decorin suppress lesion site cavitation and promote functional recovery after spinal cord injury [J]. *Sci Rep*, 2021, 11(1): 18124.
- [6] Liu X, Hao M, Chen Z, et al. 3D bioprinted neural tissue constructs for spinal cord injury repair [J]. *Biomaterials*, 2021, 272:120771.
- [7] Bonaventura G, Incontro S, Iemmolo R, et al. Dental mesenchymal stem cells and neuro-regeneration: a focus on spinal cord injury [J]. *Cell Tissue Res*, 2020, 379(3): 421-428.
- [8] Silvestro S, Bramanti P, Trubiani O, et al. Stem cells therapy for spinal cord injury: an overview of clinical trials [J]. *Int J Mol Sci*, 2020, 21(2): 659.
- [9] Papa S, Pizzetti F, Perale G, et al. Regenerative medicine for spinal cord injury: focus on stem cells and biomaterials [J]. *Expert Opin Biol Ther*, 2020, 20(10): 1203-1213.
- [10] Kiyotake EA, Martin MD, Detamore MS. Regenerative rehabilitation with conductive biomaterials for spinal cord injury [J]. *Acta Biomater*, 2020, S1742-7061(20): 30735-2.
- [11] Bartlett R D, Eleftheriadou D, Evans R, et al. Mechanical properties of the spinal cord and brain: comparison with clinical-grade biomaterials for tissue engineering and regenerative medicine [J]. *Biomaterials*, 2020, 258:120303.
- [12] Guo S, Redenski I, Levenberg S. Spinal cord repair: from cells and tissue engineering to extracellular vesicles [J]. *Cells*, 2021, 10(8): 1872.
- [13] Marquardt LM, Doullames VM, Wang AT, et al. Designer, injectable gels to prevent transplanted schwann cell loss during spinal cord injury therapy [J]. *Sci Adv*, 2020, 6(14): 1039.
- [14] Kandam S, De Berdt P, Ucakar B, et al. Human dental stem cells of the apical papilla associated to BDNF-loaded pharmacologically active microcarriers (PAMs) enhance locomotor function after spinal cord injury [J]. *Int J Pharm*, 2020, 587:119685.
- [15] Yao S, He F, Cao Z, et al. Mesenchymal stem cell-laden hydrogel microfibers for promoting nerve fiber regeneration in long-distance spinal cord transection injury [J]. *ACS Biomater Sci Eng*, 2020, 6(2): 1165-1175.
- [16] Demircan T, Hacibektaşoğlu H, Sibai M, et al. Preclinical molecular signatures of spinal cord functional restoration: optimizing the metamorphic axolotl (ambystoma mexicanum) model in regenerative Medicine [J]. *OMICS*, 2020, 24(6): 370-378.
- [17] Cao Z, Yao S, Xiong Y, et al. Directional axonal regrowth induced by an aligned fibrin nanofiber hydrogel contributes to improved motor function recovery in canine L2 spinal cord injury [J]. *J Mater Sci Mater Med*, 2020, 31(5): 40.
- [18] Moreno-Manzano V. Ependymal cells in the spinal cord as neuronal progenitors [J]. *Curr Opin Pharmacol*, 2020, 50: 82-87.