



Study on the mechanism of exercise therapy in promoting neural stem cell mobilization after stroke

Zhang Simei^{1*,#}, Jin Yaju¹, Yang Xichen¹, Sutthikiat Udommethaporn²

¹ Yunnan University of Chinese Medicine, Kunming, Yunnan Province 650500, CHINA

² School of Integrative Medicine, Mae Fah Luang University, Chiangrai 57100, THAILAND

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*Conference presenter:
Zhang Simei

Corresponding author:
Jin Yaju, Yunnan University of
Chinese Medicine, Kunming,
Yunnan, China

E-mail:
2359807454@qq.com
(Zhang Simei)

48297442@qq.com
(Jin Yaju)

yxc20000902@163.com
(Yang Xichen)

sutthikiat.udo@mfu.ac.th
(Sutthikiat Udommethaporn)

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ABSTRACT

Introduction: Stroke has become the third leading cause of death in humans, most of which are ischemic strokes. Exercise therapy is currently very popular and loved by most people. Studies have found that exercise training can promote neurogenesis, angiogenesis, synaptic plasticity, and neural stem cell proliferation and differentiation after cerebral ischemia. The Wnt/ β -catenin signaling pathway regulates cell cycle and neurogenesis.

Objective: To observe the mobilization of endogenous neural stem cells by exercise therapy in rats with cerebral ischemia and its mechanism.

Methods: Thirty SPF adult SD rats were selected, 8 rats were randomly selected as the sham operation group (sham), and the remaining rats were constructed as a middle cerebral artery occlusion (MCAO) model, and then the successfully modeled rats were randomly divided into the model group (MCAO) and exercise group (RUN), exercise group was given exercise treatment 24 hours after MCAO operation, once a day for 9 consecutive days, and model group and sham operation group were not treated. The neurological deficits of the rats were detected by behavior, the volume of cerebral infarction was detected by TTC staining, and the expressions of DCX and Wnt/ β -catenin pathway-related proteins wnt3a and β -catenin in the infarcted cortex were detected by western blot.

Results: Compared with the sham operation group, the mNSS score of the model group was significantly higher ($P < 0.001$), and compared with the model group, the mNSS score of the exercise group was lower ($P < 0.001$). The Fault score was lower ($P < 0.001$), and compared with the model group, the Foot-Fault score of the exercise group was higher ($P < 0.01$); TTC staining showed that the cerebral infarction volume in the sham operation group was 0. The volume of cerebral infarction in the group increased significantly ($P < 0.001$). Compared with the model group, the volume of cerebral infarction in the exercise group was reduced, but the difference was not statistically significant. Western blot showed that compared with the sham operation group, the protein expressions of wnt3a ($P < 0.01$) and β -catenin ($P < 0.05$) in the model group were lower, and compared with the model group, the protein expressions of DCX ($P < 0.05$) and wnt3a ($P < 0.01$) in the exercise group were higher.

Conclusion: Exercise therapy may promote the mobilization of endogenous neural stem cells in rats with cerebral ischemia by activating wnt/ β -catenin.

Keywords: exercise; cerebral ischemia; wnt/ β -catenin; neural stem cells; mobilization

Introduction

脑卒中又称脑中风，是脑部血管堵塞或破裂导致的急性脑血管病。脑卒中分为缺血性卒中和出血性卒中，缺血性卒中约占所有卒中的 69.6%，并且大多为大脑中动脉闭塞所致[1]。脑卒中具有较高的发病率和死亡率，已成为我国农村第二位和城市第三位死亡原因[2]。尽管多年来人们对脑卒中的病

因、病机等有了较深的认识，但还是缺乏较为有效且能阐明其作用机制的治疗措施。

神经干细胞是能自我更新并多向分化为神经元和胶质细胞的细胞群，其主要位于侧脑室室管膜下区 (SVZ) 和海马齿状回的颗粒下层 (SGZ) [3]。一般情况下神经干细胞处于静息状态，脑缺血后，

存在于 SVZ 的神经干细胞增殖, 迁移至缺血损伤的纹状体, 分化为神经元, 新生神经元部分替代坏死神经元, 从而修复神经功能[4]。存在于 SGZ 的神经干细胞被激活后增殖, 大多数增殖的神经干细胞可以分化成神经元, 随后, 增殖的神经干细胞会迁移至颗粒细胞层, 进而分化为神经元和神经胶质细胞, 新的神经元可以参与海马神经元的再生[5,6]。研究发现, 脑卒中后利用外源性神经干细胞移植也能最大限度恢复缺失的神经功能, 但面临伦理、肿瘤、免疫排斥等问题而遭到限制[7,8]。因此, 尽可能令脑卒中后神经干细胞增殖、迁移并分化为特定的功能性细胞是我们目前需要解决的难题。

调查研究发现脑卒中高危人群危险因素占比前三项分别为高血压、血脂异常和运动缺乏[2]。运动疗法是脑卒中后康复治疗中的重要组成部分, 其可促进神经发生、血管生成、增强树突修饰和突触可塑性^[9,10]。近来研究表明, 运动训练改善脑卒中后的神经功能与促进内源性神经干细胞迁移有关[11]。运动训练可以上调神经干细胞的数量, 有促进干细胞分化为神经元的潜能[12], 促进大鼠梗死周围运动皮层中新的树突和突触形成[13], 但其作用机制不太明确。

经典 Wnt/ β -catenin 信号通路在调节成人神经发生中发挥关键作用[14], 其与中枢神经系统密切相关, 参与神经干细胞增殖和分化的调节[15]。 β -catenin 在 Wnt/ β -catenin 信号通路中占据重要地位, Wnt 途径通过调节降解复合物来调节 β -catenin 的稳定性和最终的基因表达。Wnt3a 是 Wnt/ β -catenin 信号通路中的一个重要蛋白, 其分布广泛, 普遍认为其能激活 Wnt/ β -catenin 信号通路。因此, 本文研究运动治疗是否通过激活 Wnt/ β -catenin 信号通路从而促进脑卒中后神经干细胞动员。

Methodology

1. 试剂

兔抗 DCX 购自 abcam, 兔抗 β -catenin 购自 abcam, 山羊抗兔二抗购自 abcam, β -actin 购自索莱宝, TTC 粉末购自索莱宝, BCA 蛋白浓度试剂盒购自碧云天公司。

模型建造及分组

SPF 级雄性 SD 大鼠 30 只(180-220 g), 购自湖南斯莱克景达实验动物有限公司, 分笼饲养, 5 只 1 笼。置于 08:00-20:00 光照、20:00-08:00 黑暗的实验室中, 昼夜比为自然光控节律。室温控制于 22-26°C, 室内相对湿度 40-70%。适应性喂养大鼠 1 周左右, 待其体重增加至 250-280 g 左右开始进行大脑中动脉闭塞(MCAO)模型建造。按随机数字法将大鼠分为假手术组(sham)、模型组(MCAO)、运动组(RUN)。MCAO 造模: 腹腔注射 10% 水合氯醛进行麻醉, 仰卧位平躺在木板上, 剪去颈部正中皮肤, 撑开两边肌肉组织暴露视眼, 眼科细镊钝性分离并

活结结扎颈总动脉, 分离颈外动脉在远端死结结扎, 颈外动脉近端系根细线, 颈内动脉活结结扎, 用眼科剪于颈外动脉近心端剪一小口, 插入直径为 0.4-0.42 mm 的硅胶头线栓, 使其沿颈外动脉进入颈内动脉, 后稍系紧预备丝线, 解开颈内动脉, 将线栓从颈内动脉插入至大脑中动脉处(线栓顶端有阻塞感停止, 深度约 18-20mm), 立即系紧预备丝线, 开始计时。随后松开颈总动脉, 并缝合切口。60 min 后, 完全拔出线栓, 待大鼠清醒后放回笼中喂养。假手术组的动物除了未插线栓外, 其余手术步骤与手术组相同。

2. 治疗

MCAO 造模后 24 h, 将造模成功的大鼠随机分为模型组和运动组。MCAO 手术前两天, 对所有大鼠进行适应性训练, 10min/day, 5-8m/min, 剔除不会跑步运动大鼠, 将适应良好的大鼠编组进行后续实验。运动组大鼠在 MCAO 造模后 24 对其进行跑台运动, 1 次/天, 30min/天, 持续 9 天。模型组和假手术组正常饲养, 不作其它干预处理。

3. 神经功能缺失评分

大鼠 9 天治疗结束后, 采用 7 分制神经功能缺失评分对大鼠神经功能进行评定。

4. Foot-Fault 评分

使大鼠通过 100 cm 长的水平梯(每梯间隔 2 cm), 用于反应大鼠肢体功能缺损和运动协调能力。

5. TTC 染色

麻醉处死大鼠, 将新鲜的大脑组织切为 2mm 厚, 用 2%TTC 染液浸没切片, 37°C 避光孵育 20min 左右。

6. western blot

9 天治疗结束后, 麻醉处死大鼠, 断头取脑, 取适量梗死灶周围皮层组织, 加入裂解液裂解。用 BCA 试剂盒进行蛋白定量、配制 SDS-PAGE 胶、电泳、转膜、5%脱脂奶粉封闭 2 h、TBST 洗膜、孵一抗 1 h, 兔抗 DCX(1:1000)、兔抗 β -catenin(1:400)、兔抗 Wnt3a (1:1000) 过夜, 次日孵育二抗 1 h(1:3000), 洗膜、显影。

Results

1. 神经功能缺失评分

如图 1(a)所示, 与假手术组相比, 模型组 mNSS 得分更高($P<0.001$), 与模型组相比, 运动组 mNSS 得分较低($P<0.001$)。

2. Foot-Fault 评分

如图 1(b)所示, 与假手术组比较, 模型组 Foot-Fault 得分较低($P<0.001$), 与模型组比较, 运动组 Foot-Fault 得分较高($P<0.01$)。

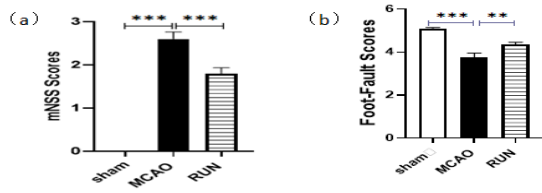


图 1 神经功能缺失评分(a)和 Foot-Fault 评分(b)(*注：p<0.05, **：p<0.01, ***:p<0.001)

3. TTC 染色

如图 2 (a)、(b)所示,假手术组梗死体积为 0,与假手术组比较,模型组梗死体积明显较高 (P<0.001),与模型组比较,运动组脑梗死体积稍微减少,但差异无统计学意义。

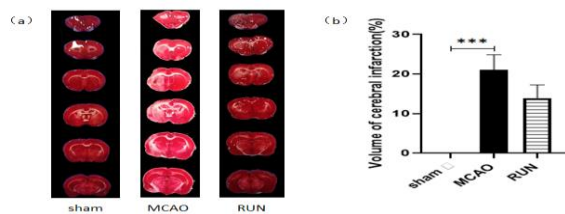


图 2 TTC 染色(a)和脑梗死体积统计(b)(*注：p<0.05, **：p<0.01, ***:p<0.001)

4. western blot

如图 3(a)、(b)所示,与假手术组相比,模型组 DCX 蛋白表达量降低,但差异不显著 (P>0.05),与模型组相比,运动组 DCX 表达量升高 (P<0.05);如图 4(a)、(b)所示,与假手术组相比,模型组 wnt3a 蛋白表达降低 (P<0.01),与模型组相比,运动组 wnt3a 蛋白表达增加 (P<0.01);图 5 (a)、(b)所示,与假手术组相比,模型组 β -catenin 蛋白表达量减少 (P<0.05),与模型组比较,运动组 β -catenin 表达较为升高,但差异无统计学意义。

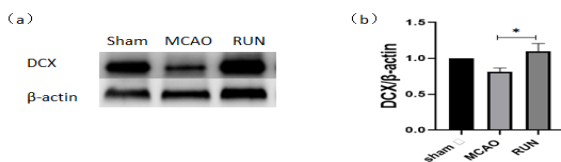


图 3 DCX 及 β -actin 显影图(a)和 DCX 蛋白表达量(b)(*注：p<0.05, **：p<0.01, ***:p<0.001)



图 4 Wnt3a 及 β -actin 显影图(a)和 wnt3a 蛋白表达量(b)(*注：p<0.05, **：p<0.01, ***:p<0.001)

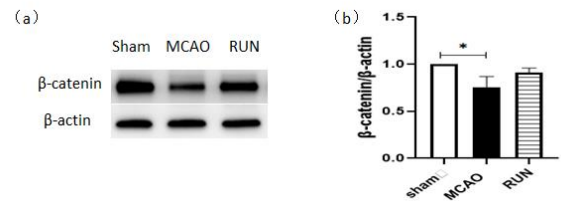


图 5 β -catenin 及 β -actin 显影图(a)和 β -catenin 蛋白表达量(b)(*注：p<0.05, **：p<0.01, ***:p<0.001)

Conclusion

在本研究中我们发现跑台运动不仅能改善脑缺血运动协调能力和神经功能缺损,而且在分子层面能促进 Wnt3a、 β -catenin 蛋白表达和迁移中的神经元 DCX 的表达,表明运动疗法能促进脑缺血大鼠神经功能恢复和神经干细胞动员,并且可能是通过激活 Wnt/ β -catenin 信号通路实现的。

Wnt/ β -catenin 信号通路中有众多分支和蛋白, Wnt3a、 β -catenin 是其中较为典型的,但仅有两个蛋白很难说明其是通过这条信号通路起作用的,我们还应该多加几个蛋白及抑制剂进行验证。另外,可以用免疫荧光或免疫组化进行 Nestin/BrdU 及 NeuN/BrdU 双染可以更好地说明脑卒中后神经干细胞的增殖、迁移和分化。这样才能使得实验设计更加严谨,实验结果更具有说服力。

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