·基础研究·

跑台运动训练对脑缺血大鼠 TGF-β1/Smad3 通路蛋白表达及细胞凋亡的影响

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【摘要】 **目的**　观察跑台运动训练对脑缺血大鼠脑缺血周边区组织中转化生长因子-β1(TGF-β1)及其受体 Smad3 蛋白表达水平及细胞凋亡的影响,探讨跑台运动训练促进大鼠脑缺血后神经功能恢复的相关机制。**方法**　取成年雄性 Sprague-Dawley(SD)大鼠 30 只,按随机数字表法分为假手术组(n=6)、模型组(n=12)和运动组(n=12)。模型组和运动组大鼠采用改良的 Longa 线栓法制备大脑中动脉闭塞(MCAO)脑缺血动物模型,假手术组大鼠手术方法同模型组和运动组,但是不插入线栓。运动组于造模成功后 24 h 采用跑台训练仪进行跑台运动训练,其余 2 组则不进行运动训练。采用修正的神经功能缺损评分方法(mNSS)评价模型组和运动组大鼠造模后第 3 天、7 天及 14 天的神经功能,并于造模后 14 d 断头取脑。采用 Western blot 法检测脑缺血周边区组织中 TGF-β1 及 Smad3 蛋白的表达情况,采用 TUNEL 法检测脑缺血周边区组织中细胞凋亡情况。**结果**　造模后第 7、14 天,运动组的 mNSS 评分分别为(7.06±0.79)分和(6.33±1.15)分,分别于模型组同时间点比较,差异均有统计学意义(P<0.05或P<0.01);运动组大鼠的 TGF-β1 及 Smad3 蛋白表达均较模型组明显增加,差异具有统计学意义(P<0.01);运动组大鼠脑缺血周边区 TUNEL 染色阳性细胞较模型组明显减少,差异具有统计学意义(P<0.01)。**结论**　跑台运动训练能够促进脑缺血后大鼠神经功能恢复,其机制可能与上调脑缺血周边区组织中 TGF-β1/Smad3 蛋白表达,进而抑制细胞凋亡有关。

【关键词】 脑缺血;　运动训练;　转化生长因子;　凋亡

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Treadmill exercise promotes the expression of TGF-β1 and Smad3 protein, inhibiting cell apoptosis after cerebral ischemia

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【**Abstract**】 **Objective**　To observe the effect of treadmill exercise on the expression of transforming growth factor-β1 (TGF-β1) and its receptor Smad3 protein as well as on cell apoptosis in the ischemic boundary zone, so as to explore how exercise promotes the recovery of neurological function after cerebral ischemia. **Methods**　Thirty adult male Sprague-Dawley rats were randomly divided into a sham group (n=6), a model group (n=12) and an exercise group (n=12). A modified Longa′s method was used to establish an animal model of cerebral ischemia by occluding the right middle cerebral artery in the rats of the model and exercise groups. Those of the sham group were subjected to the same surgical procedure except that no thread was inserted. After 24h the exercise group began treadmill training, while the other two groups were left on the treadmill without training. Modified neurological severity scores (mNSSs) were used to quantify the rats′ neurological functioning on the 3rd, 7th and 14th day after the surgery. The ischemic boundary zone tissue was then dissected to detect the expression of TGF-β1 and Smad3 protein using western blotting. Cell apoptosis was detected by TUNEL staining. **Results**　The average mNSS scores of the exercise group on the 7th and the 14th day were significantly lower than those of the model group at the same time points. The average expression level of TGF-β1 and Smad3 protein in the exercise group was significantly higher than in the model group. The number of TUNEL-positive cells in the exercise group was significantly lower than in the model group on the 14th day. **Conclusions**　Treadmill exercise can improve the recovery of neurological function after cerebral is-

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chemia. It may be partly due to upregulating the expression of TGF-β1 and Smad3 protein, which inhibit cell apoptosis in the ischemic boundary zone.

【Key words】 Cerebral ischemia; Exercise; Transforming growth factor; Smad3 protein; Apoptosis

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研究表明,运动训练可通过加强脑缺血后神经和血管网络重建促进脑缺血后运动、感觉等神经功能恢复[1],但其中的分子机制尚未完全阐明。转化生长因子 β1(transforming growth factor β1,TGF-β1)作为一种高度多效性、多功能性的生长与分化因子,可通过 TGF-β1/Smad3 信号通路抑制脑缺血后细胞凋亡及促血管再生等生物学效应促进脑缺血后功能恢复[2]。本研究采用跑台运动训练对大脑中动脉闭塞(middle cerebral artery occlusion,MCAO)脑缺血模型大鼠进行干预,观察其对脑缺血后 TGF-β1/Smad3 通路蛋白表达及脑缺血周边区细胞凋亡的影响,并探讨跑台运动训练对脑缺血大鼠的治疗作用及其可能机制。

材料与方法

一、实验动物及分组

选取雄性、健康、清洁级成年 Sprague-Dawley(SD)大鼠(由上海斯莱克公司提供,许可证号:SCXK 沪 2007-0005)30 只,体重 250~280 g,12 周龄。按随机数字表法将 30 只大鼠分为假手术组(n=6)、模型组(n=12)和运动组(n=12)。3 组大鼠均置于相同的日常环境中,常规饲养。

二、大鼠 MCAO 模型制备

采用改良的 Longa 线栓法[3]制备大鼠 MCAO 模型。大鼠经 10%水合氯醛(300 mg/kg 体重)腹腔麻醉后,仰卧固定于手术台上,取颈部正中切口,血管钳钝性分离右侧颈总动脉、颈外动脉和颈内动脉。结扎并游离一段由右侧颈外动脉主干,于右侧颈内动脉置一动脉夹,在右侧颈外动脉游离段上剪开一约 0.2 mm 的“V”字型切口,插入经预先处理过的尼龙线栓,使线栓沿着右侧颈总动脉分叉处进入右颈内动脉到达右大脑中动脉起始处,插入深度约为(18.5±0.5)mm。在手术过程中,大鼠肛温控制在(37±0.5)℃。清醒后回笼,自由进食。大鼠造模成功的分级标准参考 Longa 等的方法。造模后神经功能评分为 1~3 分的大鼠入选本次研究。假手术组大鼠手术方法同模型组和运动组,但是不插入线栓。

三、跑台运动训练

跑台运动训练采用美国哥伦布仪器有限公司制造的 exer3/6R 型电动跑台仪。各组大鼠在手术前均经过适应性跑步训练 3 d,剔除无法学会跑台运动的大鼠,并予以补充。在大鼠缺血造模成功 24 h 后,运动组大鼠给予跑台运动训练,每天 30 min,跑台运动参数设置如下:电动平板斜度为 0°;履带传输速度术前 3 d 为 12 m/min,术后第 1 天为 4 m/min,术后第 2 天为 8 m/min,术后第 3 天至第 14 天为 12 m/min。假手术组及模型组每日亦置于跑台上 30 min,但不进行跑步运动。

四、神经功能缺损评分检测

分别于 MCAO 术后 3,7,14 d 对模型组和运动组大鼠采用修订的神经功能评分(modified neurological severity scores,mNSS)方法进行评价[4]。mNSS 评分项目包括运动、感觉、反射及平衡 4 个部分,总分为 0~18 分,正常大鼠为 0 分,分值越大,表明神经功能缺损越严重。

五、Western blot 法检测

各组大鼠均于脑缺血后 14 d,神经功能缺损评分检测结束后,断头取脑缺血周边区组织约 100 mg,置于玻璃匀浆器中,加入放射免疫沉淀实验(radioimmunoprecipitation assay,RIPA)裂解液(购自碧云天公司)1 ml 进行蛋白的提取,然后采用二喹啉甲酸(bicinchoninic acid,BCA)法测定蛋白浓度。取 30 ug 蛋白,变性后于 10%十二烷基硫酸钠聚丙烯酰胺凝胶(sodium dodecyl sulfate polyacrylamide gel electrophoresis,SDS-PAGE)中垂直电泳,后电转移至聚偏二氟乙烯(polyvinylidene fluoride,PVDF)膜,10%脱脂奶粉封闭后,先后加入一抗(抗 TGF-β1 和 Smad3 蛋白抗体,1∶1000,购自美国 Santa Cruz 公司;抗 β-actin 抗体,1∶1000,购自武汉博士德公司)及二抗,孵育并洗膜后,采用电化学发光(electrochemical luminescence,ECL)液显色,X 片显影、定影。采用 Image J 图像分析测定 Western blot 条带灰度值,并与内参照 β-actin 的测定比较,计算其比值作为目的蛋白表达的相对水平,比较各组间差异。

六、细胞凋亡细胞检测

采用 TUNEL 法(TUNEL 试剂盒购自武汉博士德公司)检测脑缺血去细胞凋亡,组织用 40 g/L 多聚甲醛固定 4 h,石蜡包埋,脱蜡,入水。新鲜配制体积分数为 3%H_2O_2 室温处理 15 min。0.01 moL/L 磷酸盐缓冲液(phosphate buffered saline,PBS)洗涤 10 min×3 次,10 mmol/L 三羟甲基氨基甲烷(Tris/hydroxymethyl,

Tris/Hcl) 缓冲液加入 1 mg/ml 蛋白酶 K 稀释至 5 ug/ml,37 ℃消化 20 min,后 0.01 mol/L PBS 洗 5 min ×3 次。阳性对照加入 DNAse I,室温 30 min。然后 0.01 moL/L PBS 洗 5 min×3 次。通透液室温孵育 8 min,0.01 mol/L PBS 洗 5 min×3 次。甩去液体,擦干,按每张切片滴加 1 液和 2 液(1∶10)配成 TUNEL 反应混合液。阴性对照组只加入试剂 2 液,不加 1 液。标本上覆盖塑料盖片,置于湿盒,37 ℃标记 1 h,4 ℃过夜。然后 0.01 mol/L PBS 洗 5 min×3 次。体积分数 5%牛血清封闭液,室温 30 min,甩掉封闭液。转化剂-过氧化物酶(Peroxidase,POD)50 ul 片加至标本片上。37 ℃孵育 40 min。后 0.01 mol/L PBS 洗 5 min×3 次。DAB 显色,室温 5~10 min。双蒸水中止显色,流水冲洗。苏木素轻度复染,脱水,透明,封片,显微镜观察。

七、统计学方法

本研究采用 IBM SPSS Statistics 21.0 版统计学软件进行数据分析,数据以($\bar{x}\pm s$)表示,组间比较采用单因素方差分析,两两比较采用独立样本 t 检验。$P<0.05$为差异具有统计学意义。

结　果

一、大鼠神经功能缺损评分

造模后第 7、14 天,运动组大鼠的 mNSS 评分明显低于同时间点模型组大鼠,差异均有统计学意义($P<0.05$或$P<0.01$),造模后第 3 天,运动组大鼠 mNSS 评分和模型组大鼠无统计学意义,详见表 1。

表 1　模型组和运动组大鼠不同时间点 mNSS 评分比较(分,$\bar{x}\pm s$)

组别	只数	造模后第 3 天	造模后第 7 天	造模后第 14 天
模型组	12	8.67±1.07	8.17±1.19	7.41±1.08
运动组	12	8.08±1.08	7.06±0.79[a]	6.33±1.15[b]

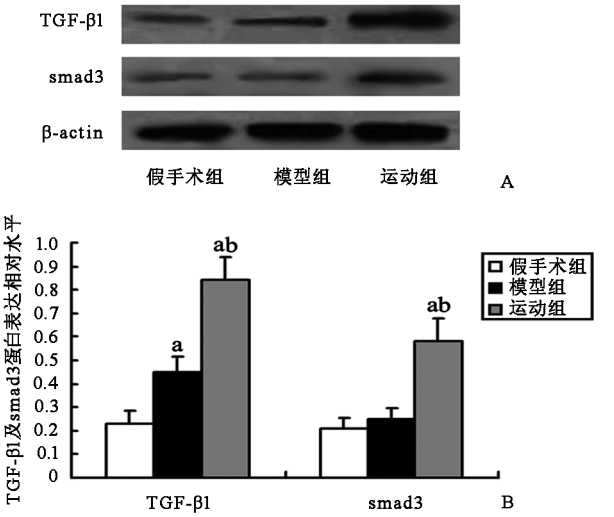
注:与模型组同时间点比较,[a]$P<0.05$,[b]$P<0.01$

二、脑缺血周边区组织中 TGF-β1 和 Smad3 蛋白表达水平

假手术组大鼠的 TGF-β1 蛋白表达呈低水平表达,造模后第 14 天,模型组大鼠 TGF-β1 表达较假手术组增加,差异具有统计学意义($P<0.01$);与模型组相比,运动训练可进一步促进 TGF-β1 表达($P<0.01$)。造模后第 14 天,假手术组大鼠 Smad3 蛋白呈低水平表达,与模型组同时间点比较,差异无统计学意义($P>0.05$);而运动组大鼠 Smad3 蛋白表达水平明显升高,与模型组同时间点比较,差异有统计学意义($P<0.01$),详见图 1。

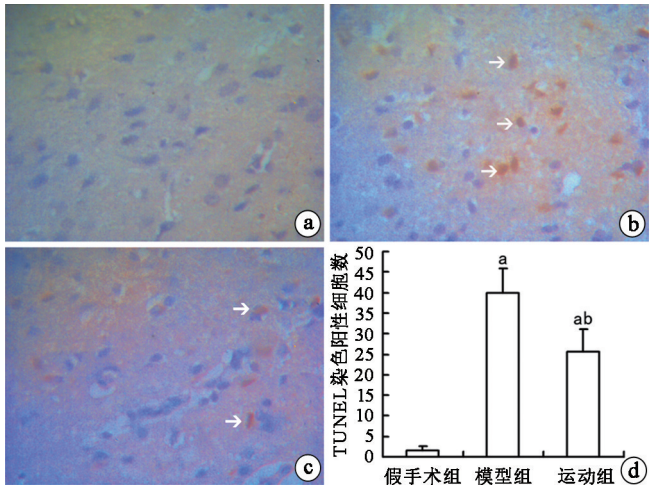
三、脑缺血周边区细胞凋亡检测

假手术组大鼠仅可见极少的 TUNEL 染色阳性凋亡细胞,模型组和运动组大鼠脑缺血周边区可见较多棕褐色 TUNEL 染色阳性凋亡细胞(图 2B、C 中白箭头所示)。运动组大鼠脑缺血周边区 TUNEL 染色阳性的凋亡细胞较模型组明显减少,差异有统计学意义($P<0.01$),详见图 2。



注:图 A 为 TGF-β1 和 Smad3 蛋白电泳图;图 B 为 3 组大鼠 TGF-β1 和 Smad3 蛋白表达情况比较。与假手术比较,[a]$P<0.01$;与模型组比较,[b]$P<0.01$

图 1　造模后 14 d 脑缺血周边区 TGF 和 Smad3 蛋白表达情况



注:图 A、B 和 C 为假手术组、模型组和运动组大鼠 TUNEL 染色代表性图片(×400 倍);图 D 为三组大鼠 TUNEL 染色阳性细胞数比较。与假手术比较,[a]$P<0.01$;与模型组比较,[b]$P<0.01$

图 2　TUNEL 染色检测脑缺血周边区细胞凋亡情况

讨　论

本研究结果显示:(1)运动组大鼠在脑缺血后 7 d 和 14 d 的 mNSS 评分较同时间点的模型组大鼠评分明显降低;(2)在脑缺血损伤发生后,在缺血周边区可见大量的 TUNEL 染色阳性的凋亡细胞,经脑缺血后早期进行跑台运动训练治疗后,缺血周边区凋亡细胞明显

减少;(3) TGF-β1 蛋白在脑缺血损伤后表达轻度升高,而早期跑台运动训练能更进一步上调脑缺血周边区 TGF-β1 及其下游 Smad3 蛋白表达。

目前,已有大量的临床研究证实运动训练治疗可促进脑缺血损伤后神经功能恢复[5-6]。脑缺血后早期运动训练介入治疗能够预防和治疗患者的运动、感觉、认知和言语等多种功能障碍,但早期过度的运动训练可能通过加剧脑缺血区炎症瀑布反应及产生过多的毒性氨基酸作用等机制使神经功能恶化[7-8]。本研究采用逐渐增加运动量的方式对脑缺血大鼠进行跑台运动训练,大鼠的适应性较好,避免了过度运动对神经功能的损伤,结果显示造模后第 7 和 14 天,运动组大鼠的神经功能缺损评分明显优于模型组,表明适当的运动训练可促进脑缺血大鼠神经功能的恢复。

缺血性脑卒中发生后,缺血中心区周围的神经细胞仍然具有代谢活力,其后续的细胞死亡以凋亡为主。已有大量文献证实可通过药物、理化因子及凋亡抑制基因转染等方式抑制脑缺血周边区细胞凋亡[9-10],改善脑缺血后神经功能缺损,从而发挥脑保护作用。因此,对脑缺血周边区神经细胞的“抢救工作”已成为抗脑缺血治疗的重要手段之一。本研究采用脉冲电动跑台仪对 MCAO 大鼠进行为期 14 d 的运动训练。结果显示运动组大鼠脑缺血周边区神经细胞凋亡数量较模型组明显减少,这与前期研究结果一致[9,11],提示运动训练减轻脑缺血后神经功能缺损可能与其抗凋亡效应有关,但运动训练抑制脑缺血后神经细胞凋亡的分子机制目前还不明确。

TGF-β1 是一种高度多效性、多功能性的生长与分化因子,能够通过特异性结合并激活酶活性的细胞表面受体参与缺血性脑损伤病理生理学过程[12]。TGF-β1 在脑组织神经元细胞及各类胶质细胞中都能产生,生理条件下,仅脑膜细胞、脉络膜丛上皮细胞中有少量 TGF-β1 表达,但在脑缺血等病理状态下,脑组织受损区域的 TGF-β1 表达水平会明显增高[12]。脑缺血损伤后经 TGF-β1 外源性注射或 TGF-β1 基因转导后可使神经元受损程度减轻,梗死面积减小,而注射 TGF-β1 拮抗剂后大脑梗死面积可显著扩大[13-14]。提示 TGF-β1 在脑缺血后具有重要的保护作用。目前已证实 TGF-β1 除了可通过抑制脑缺血后炎症反应、减弱兴奋性氨基酸毒性和促进神经及血管再生等机制发挥脑保护作用外,其还可以通过激活其下游相关的信号分子抑制脑缺血后神经细胞凋亡[15]。Smad3 是 TGF-β1 下游的 Smad 家族信号转导分子成员之一,在调控脑缺血后神经细胞凋亡过程中发挥着重要作用[2,15]。Liu 等的研究发现过表达 Smad3 基因大鼠在脑缺血后可上调抑凋亡基因 bcl-2 及下调促凋亡基因 capase-3 的表达,减少脑缺血后神经细胞凋亡[2]。本研究结果显示脑缺血损伤可诱导脑缺血周边区 TGF-β1 及 Smad3 蛋白表达,但蛋白表达的水平较低,不利于脑缺血后神经功能的恢复。而运动训练能使 TGF-β1 及 Smad3 蛋白表达明显上调,脑缺血周边区细胞凋亡较运动组明显减少。提示运动训练可通过上调 TGF-β1/Smad3 通路蛋白水平抑制脑缺血后细胞凋亡。

综上所述,脑缺血后早期跑台运动训练能够促进脑缺血后 TGF-β1 表达,提高 TGF-β1/Smad3 信号通路蛋白表达水平,抑制脑缺血后缺血周边区细胞凋亡,这可能是运动训练改善脑缺血后神经功能、发挥脑保护作用的机制之一。

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·外刊撷英·

Fractures and increased risk of suicide

BACKGROUND AND OBJECTIVE Previous studies have demonstrated a high rate of death by suicide among patients with fractures. This study evaluated the associations between fracture and the risk of suicide.

METHODS The Taiwan National Health Insurance Research Database contains information covering more than 99% of Taiwan's residents. From this database were identified all individuals 40 years of age or over with a suicide recorded as the cause of death between the years 2000 and 2011. All records identifying diagnoses of fractures and osteoporosis treated medically were reviewed. Covariates included gender, age, marital status, education, morbidity, osteoporosis, fractures, psychological comorbidity and income.

RESULTS From the database were identified 34,794 patients who had died by suicide, who were then matched with 139,176 controls. Those with fractures exhibited a higher risk of suicide (adjusted odds ratio 1.48), including all fracture sites except the wrist. Fractures of the pelvis (adjusted odds ratio 2.04), spine (adjusted odds ratio 1.53), and femoral shaft (adjusted odds ratio 1.47) had a relatively higher risk than other sites. A diagnosis of osteoporosis was not associated with an increased risk of suicide.

CONCLUSION This Taiwanese population study found that fractures are associated with an increased risk of suicide, especially hip and spine fractures.

【摘自:Chang CF, Lai EC, Yeh MK. Fractures and the increased risk of suicide: a population based, case control study. Bone Joint J, 2018, 100B:780-786.】

Risk of stroke after transient ischemic attack

BACKGROUND AND OBJECTIVE Previous studies of patients treated for a transient ischemic attack (TIA) or a minor stroke have demonstrated an increased risk of stroke within the first year. Data extending beyond one year are quite limited. This study reports on the five-year follow-up of patients treated by stroke specialists for a TIA or minor stroke.

METHODS This study involved specialized care centers in 21 countries. All subjects presented with a TIA or a minor stroke, with a modified Rankin scale (mRS) score of 0 or 1 at the initial evaluation. The acute care decisions were made by the stroke specialists, individualized to each patient. All patients were followed with interviews beginning at baseline, and at one, three and 12 months, and then yearly for five years. The primary outcome variable was a composite of death from cardiovascular diseases, nonfatal stroke or nonfatal acute coronary syndrome.

RESULTS Five-year follow-up data were available for 3,356 patients. At year five, a primary outcome event had occurred in 469 patients, corresponding to an estimated cumulative event rate of 12.9%. Of these, 50% occurred during the years two through five. At five years, a stroke had occurred in 9.5%. Death from any cause had occurred in 10.6%, and death from cardiovascular causes in 2.7%.

CONCLUSION This multicenter study of patients with minor stroke or TIA found that the rates of cardiovascular events, including stroke, were 6.4% in the first year and 6.4% in years two through five.

【摘自:Amarenco P, Lavallée PC, Monteiro Tavares L, et al. Five-year risk of stroke after tia or minor ischemic stroke. N Engl J Med, 2018, 378: 2182-2190.】