

运动训练对大鼠局灶性脑缺血后微血管新生的影响

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摘要 目的:探讨运动训练对局灶性脑缺血后微血管新生的影响。方法:55只SD大鼠随机分为假手术对照组、模型组、康复组;模型组和康复组动物以线栓法制成大鼠左侧大脑中动脉梗死模型,康复组术后1天开始进行运动训练,每周5天,共4周;其余两组常规饲养。以免疫组织化学法检测血管内皮生长因子(VEGF)、因子表达,测定微血管密度。结果:大鼠大脑中动脉栓塞后,缺血区神经元变性、坏死,VEGF和因子在缺血周边区表达明显增加,经运动训练干预后,VEGF和因子表达大量增加,微血管数目明显增加。结论:运动训练可通过促VEGF表达上调,促进微血管新生。

关键词 脑缺血;血管内皮生长因子;毛细血管新生;运动训练

中图分类号:R743,R493,R87 文献标识码:A 文章编号:1001-1242(2006)-01-0053-04

Effects of rehabilitative training on VEGF expression and angiogenesis of rats with cerebral infarction/LIU Chuanyu, MEI Yuanwu, ZHANG Xiaoqiao//Chinese Journal of Rehabilitation Medicine,2006,21(1):53—56

Abstract Objective: To evaluate the effects of rehabilitative training on VEGF expression and angiogenesis and its role in functional outcome after cerebral infarction in rats. **Method:** 55 male SD rats were divided into control group, model group and rehabilitation group randomly. The rat models of focal cerebral infarction were established with unilateral middle cerebral artery (MCA) suture occlusion method in model group and rehabilitation group. The rats in rehabilitation group were given exercising everyday, while those in other groups were reared in their original living state. VEGF expression and angiogenesis was measured in the boundary zone to brain infarction at 1d, 3d, 7d, 14d, and 28d. **Result:** It was found that VEGF expression started at 1d, and subsequently increased, reached peak value at the 3d, failed away until 14d in model group and rehabilitation group. VEGF expression was mainly detected in the boundary zone of the infarcts at every timepoint. To compare with model group, VEGF expression of rehabilitation group was significantly increased in 3d and 7d after MCAO. Compare with control group, the microvessel density(MVD) in model group and rehabilitation group were significantly increased in 14d and 28d after MCAO, especially in rehabilitation group. **Conclusion:** Rehabilitative training can increase the expression and promote the blood vessel proliferation in rats with cerebral infarction.

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Key words cerebral ischemia; vascular endothelial growth factor; rehabilitative training; angiogenesis

脑血管病,特别是缺血性脑血管病是当前社会最常见的疾病之一,其高死亡率、高致残率严重地影响了人们的生活。近年来,脑血管病的康复日益受到重视,众多研究已证实康复训练对提高脑血管患者的生存质量、减少残疾具有极其重要的作用,临床用于治疗缺血性脑血管病取得良好疗效。为进一步研究其作用机制我们应用免疫组化技术、图像分析技术,探讨运动训练对大鼠脑缺血半暗区血管内皮生长因子(vascular endothelial growth factor, VEGF)表达和毛细血管新生的影响,探讨其分子机制,为临床应用运动训练治疗急性脑梗死提供理论依据。

1 材料与方法

1.1 实验材料

1.1.1 实验动物: 成年健康雄性SD大鼠,体质量200—280g,周龄12周左右,普通级,由华中科技大学同济医学院实验动物中心提供。

1.1.2 康复训练器材: 参考李玲等^[1]的康复训练器材,采用自制滚筒式网状训练器:其中长1.0m,直径50cm的圆形网状仪器,中间被分为4个格,可同时训练4只鼠;底座有一固定架,一端有一手摇柄,可手摇按5r/min进行转动训练;该器材可训练大鼠的抓握、旋转、行走等运动。平衡木训练:采用了170cm长,2cm宽的方木棒平放在距地面7cm处,作为一个

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收稿日期:2005-06-27

平衡木让鼠在其上行走,主要训练平衡功能。转棒训练:取长150cm,直径4.5cm的木棒1根,其中点固定在3r/min的转动器上,分别向左右交替转动,可训练动态的平衡功能。

1.1.3 主要试剂: VEGF和因子免疫试剂盒均购自北京中山公司。

1.2 实验方法

1.2.1 实验动物分组: 随机取5只大鼠作为假手术组,只分离血管,不插入线拴,饲养28d取材。其余制造大鼠大脑中动脉栓塞模型(middle cerebral artery occlusion, MCAO),将造模成功、符合入选条件并存活的50只大鼠再随机分为模型组和运动康复训练组(康复组),各25只,康复组在MCAO后24h给予运动康复训练,连续训练4周;模型组常规饲养;各组再按不同时间点随机分成5组,分别于缺血后1d、3d、7d、14d、28d取材。

1.2.2 MCAO 动物模型的建立: 参照廖维靖和 Longa 等^[2-3]的大鼠大脑中动脉缺血模型造模方法。大鼠腹腔注射10%的水合氯醛(350mg/kg体重)麻醉,仰卧固定于手术台上,颈部正中切口,长约2cm,暴露分离左侧颈总动脉(CCA)和颈外动脉,结扎颈外动脉,在距CCA分叉近端0.5—0.6cm处结扎CCA,结扎颈外动脉根部及分支。在颈总动脉近分叉处剪一小口,经此插入直径0.20mm头端圆钝的渔线,经颈总动脉分叉处通过颈内动脉入颅至大脑中动脉的起始部以阻断大脑中动脉血流,插入深度为19.0±0.5mm。结扎颈总动脉,缝合皮肤。动物苏醒后表现为提尾时右侧前肢内收屈曲;同侧Horner征;爬行时向右划圈;站立时右侧倾倒。凡具有上述四项体征者列入研究对象,共用大鼠95只,有50只符合入选条件并存活到规定时间。

1.2.3 康复训练方法: 康复组大鼠术后24h开始康复训练,每天置于滚筒式网状训练器内转动训练20min,平衡木、转棒上行走训练20min,共40min;每周5次,共进行4周。

1.2.4 标本采集: 处死前将大鼠深麻醉后用生理盐水200ml快速左心室灌注冲洗,再用4%多聚甲醛(pH7.4,0.01M PB配置)300ml先快后慢灌注固定,灌注时间45—60min,灌注完毕立即断头取脑,于前囟前2mm到后囟取脑,4%多聚甲醛后固定(不超过

24h),蒸馏水浸泡4h,常规乙醇脱水、二甲苯透明、浸蜡、包埋。石蜡切片机连续冠状切片(片厚7μm),每隔100μm连续取3张,相邻切片分为2套分别用于VEGF和因子的免疫组化测定。

1.2.5 VEGF 和因子免疫组织化学检测方法: 采用免疫组化SAB法检测VEGF和因子,操作步骤严格按说明书进行。

1.2.6 VEGF 图像分析: 每张切片取梗死周边区不同的3个视野进行照像(Olympus光学显微镜,10×20),用HMIAS-2000型彩色图像分析系统测定梗死周边区(本实验指梗死外侧边皮质区域1mm范围内)免疫表达物的平均光密度值(OD值),取3个视野的均值作为该切片结果参与统计分析。因在每个标本中取3张切片,故每个时间点有15张切片结果。

1.2.7 微血管密度 (microvessel density, MVD) 测定: MVD计数按Weidener等^[4]方法进行,凡因子免疫组化染色成棕黄色的单个内皮细胞或内皮细胞簇作为一个血管计数,每张切片在同一皮层缺血区周边选择4个高倍(×400)视野进行微血管计数,参照Weidener计数方法,计算出每mm²面积内微血管的数量,即微血管密度,然后求其均值,每个时间点每组有15张切片结果。

1.3 统计学分析

每组样本结果用均数±标准差($\bar{x} \pm s$)表示,组间比较用方差分析,组间两两比较用q检验,应用SPSS10.0统计软件, $P < 0.05$ 为有显著性意义。

2 结果

2.1 VEGF 免疫组化及图像分析结果

假手术组动物仅见极弱VEGF表达,模型组和康复组大鼠缺血边缘区脑组织在缺血1d时VEGF开始表达,3d达高峰,此后开始减少,7d时仍有表达,14d表达基本恢复正常,其对侧非缺血脑组织自缺血1d后亦开始有少量VEGF表达,但明显少于病变侧,7d时表达恢复正常。各时间点VEGF的表达主要集中在梗死灶周围,梗死灶中心仅见少量VEGF蛋白表达。表达细胞主要为神经胶质细胞,其次为神经细胞及血管。3d和7d时康复组VEGF在缺血周边区的表达明显高于模型组,差异有显著性意义,见表1。

表1 各组大鼠缺血周边区 VEGF 表达的比较

组别	鼠数	缺血1d	缺血3d	缺血7d	缺血14d	缺血28d (OD值, $\bar{x} \pm s$)
假手术组	5					0.076±0.008
模型组	25	0.146±0.011 ^①	0.212±0.020 ^①	0.201±0.017 ^①	0.075±0.010	0.073±0.009
康复组	25	0.143±0.015 ^①	0.257±0.022 ^{①③}	0.225±0.019 ^{①②}	0.080±0.011	0.078±0.010

①与假手术对照组比较 $P < 0.01$;与模型组比较② $P < 0.05$,③ $P < 0.01$

2.2 微细血管密度

因子免疫组织化学染色结果显示,微血管形态不规则,管腔由染成棕黄色的内皮细胞围成。康复组动物缺血14d和28d时缺血周边区可见较多散在的单个内皮细胞,而其它组动物相应部位仅见少见散在的单个内皮细胞。微血管计数:缺血14d和28d时

缺血周边区模型组明显多于假手术组,差异有显著性意义;而康复组缺血14d和28d时缺血周边区微血管计数明显多于假手术组和相应时间的模型组,差异有显著性意义。各组动物缺血周边区微血管密度比较见表2。

表2 各组大鼠缺血周边区微血管密度比较

组别	缺血1d	缺血3d	缺血7d	缺血14d	(血管数/mm ² , $\bar{x}\pm s$)
假手术组					55.7±23.5
模型组	61.6±19.6	62.1±25.6	68.9±23.4	74.6±24.3 ^①	80.1±24.8 ^①
康复训练组	60.4±21.7	63.4±28.7	76.2±25.1	92.8±24.5 ^{②③}	99.6±26.6 ^{②③}

与假手术对照组比较① $P<0.05$,② $P<0.01$;③与模型组比较 $P<0.05$

3 讨论

研究表明,脑缺血、缺氧可诱导VEGF及其受体的表达,并激活血管新生机制,新生血管形成可在一定程度上减轻神经功能的缺失,微血管新生的范围与程度直接关系到缺血边缘区血流的改善,影响神经元生理功能的恢复,从而决定了患者的预后,尤其是在梗死周围半暗带中新生血管的数量与中风患者的存活率直接相关^[5-6]。Krupinski等^[7]定量分析了10例脑卒中死亡患者脑组织中新生血管密度与生存时间关系,发现有9例患者缺血半球内微血管密度明显增加,以缺血半暗区增加更为显著,与患者的生存时间呈正相关。

血管再生是一个极其复杂的过程,涉及内皮细胞分裂、血管基底膜及细胞外基质的降解和内皮细胞迁移等;新血管的形成来源于先前存在的血管的内皮细胞增殖,而内皮细胞增殖有赖于VEGF的刺激^[8]。VEGF是强效的有丝分裂原,可直接或间接地影响血管再生的各个环节,在人和动物胚胎发育阶段呈广泛性高水平表达,介导生理过程的大血管形成和微血管再生。VEGF为分泌性蛋白,作用于特异性受体,从而保护脑毛细血管内皮细胞,防止毛细血管消失,同时刺激内皮细胞增殖,血管新生,神经功能迅速恢复^[9-10]。最近的研究表明,VEGF还可通过抑制脑缺血后神经细胞凋亡、调节离子通道、促进神经发生、直接神经营养等机制促进神经功能恢复^[11-12]。

既往的研究发现,脑缺血后血流增加可诱导VEGF的大量表达^[13-14],运动康复训练具有改善侧枝循环、增加脑缺血后脑血流量的作用^[1],因此从理论上讲,具有诱导VEGF表达的可行性,本实验结果显示,在脑缺血1d时VEGF开始表达,3d达高峰,此后开始减少,14d时表达基本恢复正常,其对侧非缺血脑组织自缺血1d后亦开始有少量VEGF表达,7d时表达恢复正常。各时间点VEGF的表达主要集中在梗死灶周围,梗死灶中心仅见少量VEGF蛋白表达。

3d和7d时康复组VEGF在缺血周边区的表达明显高于模型组,差别有显著性意义,这一结果进一步证实了上述观点。

对脑缺血后微血管密度的分析结果显示,与假手术组相比缺血14d和28d时缺血周边区模型组和康复组微血管计数明显多于假手术组,而康复组缺血14d和28d时皮层缺血周边区微血管计数又明显多于相应时间的模型组,说明在脑缺血后在缺血周边区微血管有代偿性增生,而运动康复训练对这种增生有促进作用。实验结果还显示,脑缺血后微血管明显增生的时间在缺血后14d和28d,明显晚于VEGF表达的时间,考虑微血管的增生可能为VEGF促进的结果。

局灶脑缺血后在脑梗死周边区VEGF的表达和微血管的增生对改善梗死周边区的微循环、促进功能恢复具有重要意义,而运动康复训练对这一过程有明显的促进作用,这可能是运动康复训练促进脑缺血后功能恢复的一个重要机制。

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