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UCF-101 对大鼠局灶性脑缺血再灌注后 JNK 和 ERK 活性的影响 *

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摘要 目的:探讨 UCF-101 对局灶性脑缺血再灌注大鼠脑内 c-Jun 氨基末端激酶(JNK)和胞外信号调节酶(ERK)活性的影响,进一步探讨 UCF-101 对局灶性脑缺血再灌注损伤脑保护作用的机制。**方法:**采用大脑中动脉线栓法(MCAO)建立大鼠局灶性脑缺血再灌注模型,随机分为假手术组,缺血再灌注组,UCF 组,应用 TTC 检测大鼠脑梗死体积,TUNEL 法检测神经元凋亡,Western blot 检测 ERK 和 JNK 的活性。**结果:**UCF-101 可下调脑缺血再灌注大鼠脑组织 JNK 蛋白的活性,上调 ERK 蛋白的活性,并降低梗死体积、坏死和凋亡细胞数。**结论:**UCF-101 对大鼠局灶性脑缺血再灌注损伤有保护作用,抑制 JNK 凋亡通路、促进 ERK 生存通路,从而减轻细胞凋亡是其脑保护机制之一。

关键词: UCF-101; 脑缺血再灌注; 胞外信号调节酶; c-Jun 氨基末端激酶

中图分类号: Q95-3; R743 **文献标识码:** A **文章编号:** 1673-6273(2015)19-3613-03

The Effect of UCF-101 on the Activation of JNK and ERK after Focal Cerebral Ischemia-reperfusion Injury in Rats*

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ABSTRACT Objective: To investigate the neuroprotective effect of 5-[5-(2-nitrophenyl)furfuryliodine]-1,3-diphenyl-2-thiobarbituric acid (UCF-101) on activation of C-Jun NH₂-terminal kinases (JNK) and extracellular-regulated protein kinases (ERK) after cerebral ischemia-reperfusion injury in rats. **Methods:** The focal cerebral ischemia models of Wistar rats were established by the right middle cerebral artery occlusion (MCAO) with thread occlusion methods. Reperfusion began 1h after the right middle cerebral artery occlusion. Rats were randomly divided into sham operated group, ischemia-reperfusion group and UCF-101 treated group. Brain infarct volume was assessed by 2, 3, 5-triphenyl tetrazolium chloride and TUNEL staining was utilized to evaluate the amount of apoptosis. In addition, expressions of protein JNK and ERK were examined by Western blot analysis. **Results:** UCF-101 treatment significantly decreased cerebral infarct size and TUNEL-positive cells in hippocampus neurons. Furthermore, UCF-101 treatment decreased JNK and increased ERK expression. **Conclusion:** UCF-101 treatment has neuroprotective effects against cerebral ischemia-reperfusion injury in rats, and that this may be associated with differential regulation of ERK and JNK expression.

Key words: UCF-101; Cerebral ischemia-reperfusion; ERK; JNK

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前言

近年来研究者发现一种重要的线粒体丝氨酸蛋白酶 O-mi/HtrA2 特异抑制剂 UCF-101,可以减轻脑、心、肾缺血再灌注损伤发挥保护作用^[1-10],但是 UCF-101 在局灶性脑缺血再灌注后的神经保护作用及其分子机制还不清楚。有研究表明丝裂原激活蛋白激酶 (mitogen-activated protein kinases, MAPK) 信号转导途径参与了脑缺血损伤与修复机制。MAPK 家族主要有 3 个成员:即 p38MAPK (p38 mitogen activated protein kinases), 胞外信号调节酶 (extracellular signal regulated protein kinases, ERK), c-Jun 氨基末端激酶 (c-jun N-terminal kinases, JNK)。

ERK 通路能够促进细胞存活、抑制细胞凋亡,而 JNK 通路主要在促细胞凋亡中发挥作用^[11-13]。本实验以建立的大鼠 MCAO 模型为研究对象,观察 UCF-101 对大鼠脑缺血再灌注后脑梗死体积及凋亡细胞数的影响,应用 Western blot 检测 pJNK 及 pERK 的表达水平,探讨 UCF-101 在脑缺血再灌注损伤中保护机制。

1 材料与方法

1.1 实验动物

45 只雄性 10 周龄 Wistar 大鼠,体重约 250-300 g,购自哈尔滨兽研研究所。饲养温度 22~24 ℃,湿度 50~70%,大鼠可自由走动,饮水,摄取标准饲料。

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1.2 实验分组

随机分为3组:(1)假手术组:除不插线栓外,其余步骤同缺血组;(2)缺血组:常温条件下缺血2h后再灌注6h;(3)UCF组:于再灌注前10分钟腹腔内注射UCF-101 10 mg/kg;其余各组注射等量生理盐水。

1.3 模型的制备

采用改良的Zea Longa^[14]线栓法建立大鼠左侧MCAO/R模型。参照Longa神经功能缺失评分标准,0分:无神经功能缺损症状;1分:左前肢不能完全伸直,提尾实验阳性;2分:行走时向左转圈;3分:行走时向左侧倾倒;4分:意识丧失,不能自主行走。以大鼠麻醉清醒后1~4分为有效模型。

1.4 脑梗死体积的测定

脑缺血再灌注6h后,将大鼠断头取脑。切成等距的冠状脑片,避光,放入37℃的TTC溶液中染色30 min,甲醛固定24 h,用计算机图像分析软件测量脑梗死体积,用梗死体积占半球体积百分比(V%)表示。

1.5 TUNEL 法观察海马神经元凋亡

采用TUNEL试剂盒(武汉博士德生物技术公司),实验步骤按试剂盒提供的操作手册进行。每只动物选取5张切片,每张切片选取8个凋亡细胞密集区(200×),计算每个视野中的阳性细胞数,其均数作为该例动物的凋亡细胞数。

1.6 蛋白印记法

将脑组织分别加入1 mL细胞裂解液研磨,匀浆离心后,去上清液用Bradford蛋白浓度测定试剂盒检测蛋白含量。取50

μg样本经十二烷基硫酸钠-聚丙烯酰胺凝胶电泳及转膜后,蛋白即转至PVDF膜。20%脱脂奶粉室温封闭2 h,加一抗:磷酸化和总的pJNK、pERK、β-actin兔抗鼠多克隆抗体(北京博奥森公司),4℃过夜,随后TBST洗膜3次,加二抗过氧化物酶标记的羊抗兔IgG(北京中杉生物技术公司),室温孵育2 h,ECL显色。用Quantiscan 4.4分析软件测定每条带的光密度值。以β-actin为内参照。将相对光密度值作为统计学分析数据。

1.7 统计学分析

采用SPSS 17.0统计软件包对数据进行处理,实验数据用 $\bar{x} \pm s$ 表示,两组间比较采用t检验分析,P<0.05表示有统计学意义。

2 结果

2.1 各组相对梗死体积的比较

假手术组的脑组织切片全部红色,未见梗死体积,缺血组大鼠右侧额、顶叶皮质及纹状体可见明显的白色梗死灶,相对梗死体积为 $28.0 \pm 3.0\%$,与缺血组比较,UCF治疗组大鼠右侧白色梗死灶明显缩小,相对梗死体积为 $16.3 \pm 3.1\%$,有显著性差异(P<0.01)。

2.2 TUNEL 凋亡细胞

假手术组偶见TUNEL阳性细胞,缺血组海马区可见大量凋亡细胞,凋亡细胞数是 76.2 ± 12.6 。UCF组凋亡细胞数 48.6 ± 10.3 。与缺血组相比,UCF治疗组TUNEL阳性细胞数明显减少(P<0.01)。

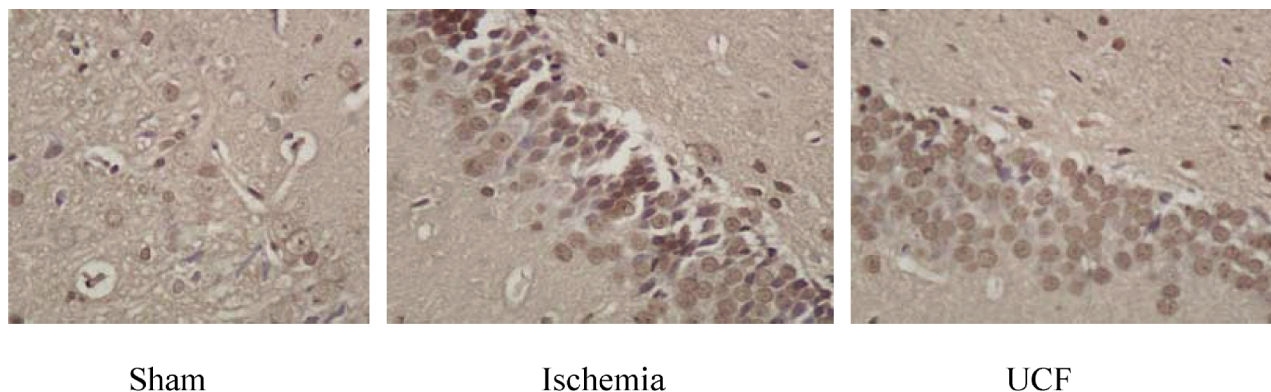


图1 各组海马区神经元凋亡的比较(TUNEL法, × 200)

Fig. 1 Comparison of neuronal apoptosis of hippocampus in all groups(TUNEL, × 200)

2.3 Western Blot 检测

如图所示,假手术组检测到少量pJNK蛋白的表达,与假手术组相比,缺血组海马区pJNK的表达水平明显增高(P<0.05),与缺血组比较,UCF组可减低海马区pJNK的表达水平(P<0.05)。假手术组检测到少量pERK蛋白的表达,与假手术组相比,缺血组海马区pERK的表达水平明显增高(P<0.05),与缺血组比较,UCF组可增加海马区pERK的表达水平(P<0.05)。

3 讨论

脑缺血/再灌注可引起海马等缺血敏感区的神经元凋亡^[15]。本研究结果显示,脑缺血组大鼠梗死体积和海马区凋亡细胞明显增加,UCF治疗组能减少大鼠脑梗死体积和海马区神经元凋亡,对脑缺血再灌注损伤具有一定的脑保护作用。我们的实验结果与Althaus等的研究结果基本相同^[4,5]。

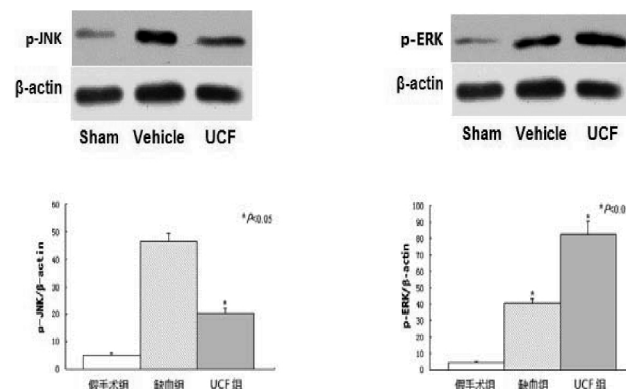


图2 各组p-JNK、pERK蛋白的表达(Western blotting法)

Fig. 2 The expression levels of p-JNK, pERK protein (Western blotting)

丝裂原激活蛋白酶(MAPK)是一组丝氨酸/苏氨酸蛋白激酶,控制细胞增值、分化、凋亡、存活等生理功能和过程,主要有ERKs、JNKs/SAPKs、P38MAPK和MEK5/ERK5等4条通路^[9],其中研究最多的是前3条通路。目前,ERK通路在脑缺血损伤中的作用存在争议。但多数学者认为,ERK信号通路是细胞的生存通路,抑制细胞凋亡中发挥重要的作用。JNK信号通路是MAPK中重要的通路,也是存在于神经细胞中的信号转导途径,主要促细胞凋亡。抑制JNK及激活ERK信号通路均可不同程度地减少缺血海马细胞凋亡,保护海马功能^[17-20]。本实验可以观察到,相对于假手术组,脑缺血再灌注损伤组海马区pJNK和pERK1/2的表达水平显著增多,说明JNK及ERK1/2通路被激活,而UCF治疗组海马区pJNK的表达水平降低,pERK的表达水平升高,表明UCF-101可能通过下调活化JNK的表达水平、上调活化ERK1/2的表达水平发挥保护海马区神经元。

综上所述,UCF-101对脑缺血再灌注损伤有脑保护作用,其作用机制与降低pJNK的表达水平以及增加pERK的表达水平有关。UCF-101可能通过抑制JNK通路和促进ERK通路抑制神经细胞凋亡,发挥神经保护作用。

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