高温高湿环境颅脑火器伤后兔脑 p38 MAPK 变化

黄柒金,徐如祥,柯以铨(南方医科大学珠江医院神经外科,广东 广州 510282)

摘要:目的 研究 p38 MAPK 在高温高湿环境下颅脑火器伤后活化及其原因。**方法** 采用高温高湿颅脑枪伤模型,新西兰大白兔 30 只,随机分成常温 [(22.0±0.5)℃、RH 50%]对照组,高温高湿 [(39.0±0.5)℃、RH 80%~85%]枪伤后受热 10 和 30 min,1 h、1.5 h、2 h 组,每组 5 只。采用 Western blot 检测脑匀浆 p38 MAPK 活性,应用化学发光和 X 线片显示,凝胶图像分析仪半定量分析。**结果** 颅脑枪伤受高温高湿环境作用脑皮质和下丘脑的 p38 MAPK 的活性受湿热后迅速增高,于受热 1 h 达高峰,随后下降。下丘脑的 p38 MAPK 活性比脑皮质高。**结论** 高温高湿环境颅脑火器伤后 p38 MAPK 活性早期明显升高,参与了脑的继发性损害过程。

关键词:高温高湿;颅脑火器伤;p38 丝裂原激活蛋白激酶

中图分类号:R651.1 **文献标识码:**A **文章编号:**1000-2588(2005)09-1155-03

Changes of brain p38 MAPK in rabbits with craniocerebral gunshot injury in hot and humid environment

HUANG Qi-jin, XU Ru-xiang, KE Yi-quan

Department of Neurosurgery, Zhujiang Hospital, Southern Medical University, Guangzhou 510282, China

Abstract: Objective To study the activation of p38 mitogen-activated protein kinase (MAPK) in the brain of rabbits after craniocerebral gunshot injury in a hot and humid environment (HHE) and explore its possible mechanism. **Methods** Craniocerebral gunshot injury model was established in 30 New Zealand white rabbits, which were subsequently exposed to environment of normal temperature (at 22.0±0.5 ℃ with relative humidity of 50%) or HHE at 39.0±0.5 ℃ with relative humidity of 80%-85% for 10 min, 30 min, 1 h, 1.5 h, and 2 h groups, respectively, with 5 rabbits in each group. p38 MAPK activity in the brain tissues of the rabbits following the injury and environmental exposure were detected by Western blotting and analyzed semi-quantitatively by Bio-Profil gel image analysis system. **Results** p38 MAPK activity in the cortex and hypothalamus was significantly elevated following gunshot injury and HHE exposure, reaching the peak level at 1 h of HHE exposure and then decreased. p38 MAPK activity was significantly higher in the hypothalamus than in the cortex. **Conclusion** p38 MAPK activity increases in the early stage following craniocerebral gunshot injury and HHE exposure in rabbits, the mechanism of which might involve the secondary brain insult.

Key words: high temperature and humidity; craniocerebral gunshot injuries; p38 mitogen-activated protein kinase

脑损伤后继发性神经细胞死亡加重了原发脑损伤,是影响脑损伤康复的一种重要因素。近年来已证明了 p38 MAPK (MAPK,mitogen-activated protein kinase,丝裂原激活蛋白激酶)信号转导通路参与了脑损伤后的继发性神经细胞损害作用过程[1,2]。本研究探讨 p38 MAPK 在高温高湿环境下颅脑火器伤后活性变化及作用。

1 材料与方法

1.1 主要仪器与材料

仿真模拟气候舱由南方医科大学热带医学研究所研制,可调节温度、湿度、风速、辐射强度等。Powlab/8sp 生理记录仪。PMSF、Aprotinin、EDTA 和 EGTA 均为 Sigma 产品。Phospho-p38 MAPK antibody 为 New England BioLabs 产品

1.2 实验动物分组与处理

参照猫脑枪弹伤模型[3]制作兔颅脑枪弹伤模型。Powlab/8sp生理记录仪动态监测生命体征变化。选用体质量 2.3±0.3 kg 的新西兰大白兔 30 只随机分成,常温对照组:置于温度(T)(22.0±0.5) ℃、相对湿度(RH)50%的仿真模拟气候舱中。高温高湿枪伤组:颅脑枪弹伤后即置于 T(39.0±0.5) ℃、RH 80%~85%的仿真模拟气候舱中,分别受湿热 10 min,30 min,1 h,1.5 h,2 h 组。每组 5 只,共 6 组。各组动物受热处理处死,取脑皮质、下丘脑于液氮速冻,后置于–80 ℃贮存,备 p38 MAPK 活性检测。所用动物均由南方医科大学实验动物中心提供。

1.3 p38 蛋白激酶活性的检测[4-6]

收稿日期:2004-10-30

基金项目:军队九五重点课题(012054)

Supported by Key Research Project of the 9th Five-year Plan of PLA (012054)

作者简介:黄柒金(1967-),男,医学博士,主治医师,讲师,电话:13503020098, E-mail: huangqijin@hotmail.com

采用 Western 印迹法。取 100 mg 脑组织,加 500 μl 含 PMSF 和 Aprotinin 的缓冲液,冰浴匀浆、超声破碎,取 300 μl 匀浆,加入 SDS 凝胶加样缓冲液 240 μl,再加入 DTT 60 μl 混匀。经煮沸离心,取上清,测定蛋白浓度[7]。等量匀浆上样,进行 10% SDS-PAGE。电泳结束电转仪将蛋白转移到硝酸纤维素膜(NC 膜)。NC 膜经过封闭、漂洗后,分别加入一抗(Phospho-p38 MAPK antibody)二抗孵育。应用化学发光和 X 线片显示。凝胶图象分析仪分析。

1.4 统计学处理

用 SPSS 统计软件包的多个样本均数比较的方差分析方法进行统计学处理,以 $P<0.05$ 为判断标准。

2 结果

2.1 动物生命体征的变化

受高温高湿环境的作用加重了颅脑枪弹伤后生命体征的紊乱,加快了呼吸、循环、体温调节的衰竭,高温高湿恶劣环境的影响比单纯枪伤更加明显。

2.2 高温高湿枪伤后 p38 MAPK 的活性变化 (图 1~3)

如图 1~3 所示,颅脑枪伤受高温高湿环境作用10 min 后脑皮质的 p38 MAPK 的活性为对照组的 2.08 倍($P<0.01$),30 min 继续升高,达 3.86 倍($P<0.01$),1 h 达高峰,5.23 倍($P<0.01$),随后下降,1.5 h 时为 3.67 倍($P<0.01$),2 h 时为 2.37 倍($P<0.01$),仍显著高于对照组。颅脑枪伤受高温高湿环境作用 10 min 后下丘脑的 p38 MAPK 的活性为对照组的 2.21 倍($P<0.01$),30 min 继续升高,达 3.98 倍($P<0.01$),1 h 达高峰,5.63 倍($P<0.01$),随后下降,1.5 h 时为 3.74 倍($P<0.01$),2 h 时为 2.65 倍($P<0.01$),仍显著高于对照组。下丘脑的 p38 MAPK 活性变化比脑皮质大,但差异无统计学意义。

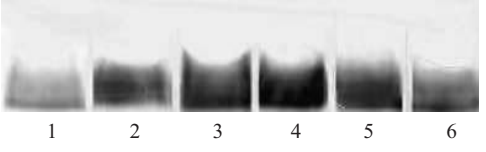

图 1 脑皮质 p38 MAPK Western blot 结果

Fig.1 Western blotting of p38 MAPK in rabbit cortex following craniocerebral gunshot injury

Lane 1: Normal temperature group; Lane 2: 10 min of hot and humid environment (HHE) exposure; Lane 3: 30 min of exposure; Lane 4: 1 h of HHE exposure; Lane 5: 1.5 h of HHE exposure; Lane 6: 2 h of HHE exposure.

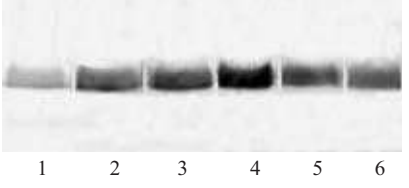

图 2 下丘脑 p38 MAPK Western blot 结果

Fig.2 Western blotting of p38 MAPK in rabbit hypothalamus following craniocerebral gunshot injury

Lane 1: Normal temperature group; Lane 2: 10 min of hot and humid environment (HHE) exposure; Lane 3: 30 min of exposure; Lane 4: 1 h of HHE exposure; Lane 5: 1.5 h of HHE exposure; Lane 6: 2 h of HHE exposure.

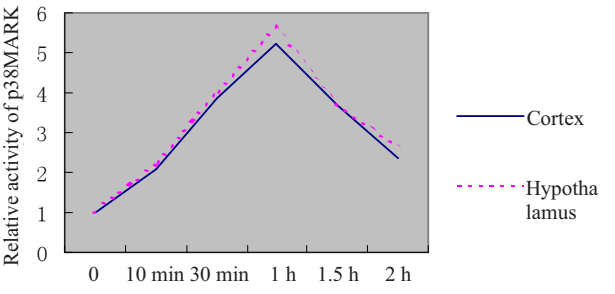

图 3 高温高湿颅脑枪伤组 p38MAPK 相对活性的变化

Fig.3 Changes in p38 MAPK relative activity during HHE exposure following the gunshot injury

3 讨论

本研究采用颅脑枪弹伤模型,观察了高温高湿颅脑火器伤后不同时间点伤侧脑皮质、下丘脑的 p38 MAPK 活性的变化,发现颅脑枪伤后高温高湿环境可显著增高脑皮质和下丘脑的 p38 MAPK 活性,呈时间依赖性,但受高温高湿较长时间后 p38 MAPK 活性反而逐渐下降。下丘脑的 p38 MAPK 活性变化比脑皮质高可能是与下丘脑是体温调节中枢,对其周围的温度升高敏感,反应性表现为细胞内应激增强有关。与文献报道经缺氧处理的培养脑神经细胞[8,9],单纯受热损伤后的 Raw264.7 细胞[10],p38 MAPK 的活性升高,引起细胞的凋亡,细胞的存活率明显下降相一致。

p38 MAPK 通路是近年来研究中最引人注目的蛋白激酶级联通路,是目前已确定的 MAPKs 四条通路之一,该通路参与细胞生长、发育、分裂及细胞间的功能同步等多种细胞生物学过程,在外界应激刺激,如热损伤、缺氧等作用下 p38 MAPK 被磷酸化活化,并通过磷酸化活化转录因子调控特定基因的表达,从而将信号从细胞外传导到细胞核,参与不良刺激后诱导细胞的凋亡的作用[11,12]。高温高湿颅脑火器伤存在着复杂的病理生理变化,颅脑火器伤后自身的调节机制减弱,加上过高的湿热环境作用,导致下丘周围体液温度升高,使下丘脑体温调节、血管舒缩和出汗调

节中枢等功能发生障碍,影响了它对交感神经、皮肤血管舒缩和排汗的调节,导致体热蓄积;过热的脑组织常处于缺氧状态,热损伤[10]、缺氧[13]这些强烈环境应激,使得 p38 MAPK 迅速被磷酸化活化,从而将信号从细胞外传导到细胞核, 参与高温高湿颅脑火器伤后诱导神经细胞的死亡。

因此本实验表明高温高湿环境可能通过 p38 MAPK 通路的迅速活化机制引起颅脑火器伤的继发性损害,是颅脑火器伤后的二次创伤。所以了解高温高湿的损伤分子、信号通路机制,对提高高温高环境下颅脑火器伤的二次创伤的防治是十分重要的。

参考文献:

[1] Nick J A, Avdi N J, Gerwins P, *et al*. Activation of a p38 mitogen-activated protein kinase in human neutrophils by lipopolysaccharide[J]. J Immunol, 1996, 156: 4867-975.

[2] Jiang Y, Li ZJ, Schwarz EM, *et al*. Structure function studies of p38 mitogen-activated protein kinase: Loop 12 influences substrate specificity and autophosphorylation, but not upstream kinase selection[J]. J Biol Chem, 1997, 272: 11096-102.

[3] Carey ME, Saran GS, Farrell JB, *et al*. Experimental missle wound to the brain[J]. J Neurosurg, 1989, 71: 754-64.

[4] Ben R, Levy S. Nuclear export of the stress-activated protein kinase p38 mediated by its substrate MAPKAP kinase-2 [J]. Curr Biol, 1998, 8: 1049-57.

[5] Huang C. p38 kinase mediates UV- induced phosphorylation of p53 protein at serine 389[J]. J Biol Chem, 1999, 274: 12229-35.

[6] McDonald DR. β-amyloid fibrils activate parallel mitogen-activated protein kinase pathways in microglia and THP1 monocytes [J]. J Neurosci, 1998, 18: 4451-60.

[7] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding [J]. Anal Biochem, 1976, 72: 248-54.

[8] Nakano A, Baines CP, Kim SO, *et al*. Ischemic preconditioning activates MAPKAPK2 in the isolated rabbit heart: evidence for involvement of p38 MAPK[J]. Circ Res, 2000, 86: 144-151.

[9] Barancik M, Htun P, Strohm C, *et al*. Inhibition of the cardiac p38-MAPK pathway by SB203580 delays ischemic cell death[J]. J Cardiovasc Pharmacol, 2000, 35: 474-83.

[10]张 璐, 张 琳, 姜 勇, 等. P38 蛋白激酶在热损伤诱导单核细胞 Raw264.7 凋亡中的作用 [J]. 中国生物化学与分子生物学报, 1999,15: 987-91.
Zhang L, Zhang L, Jiang Y, *et al*. The role of p38 protein kinase in heat-induced Raw cell apoptosis [J]. Chin J Biochem Mol Biol, 1999,15: 987-91.

[11] Luo Y, Kokkonen GC, Hattori A, *et al*. Dopamine stimulates redox -turosine signaling and p38 MAPK in activation of astrocytic C6-D2L Cells[J]. Brain Res, 1999, 850: 21-38.

[12] Armstrong SC, Delacey M, Ganote CE. Phosphorylation state of hsp27 and p38 MAPK during preconditioning and protein phosphatase inhibitor protection of rabbit cardiomyocytes[J]. J Mol Cell Cardiol, 1999, 31: 555-67.

[13] Wu DC, Ye W, Che XM, *et al*. Activation of mitogen-activated protein kinases after permanent cerebral artery occlusion in mouse brain[J]. J Cereb Blood Flow Metab, 2000, 20: 1320-30.

(上接 1154 页)

定疾病状态下发生改变的磷酸化蛋白,为研究细胞内磷酸化蛋白信号转导通路打下坚实的基础。

参考文献:

[1] 孙 玉. 蛋白质的可逆磷酸化及其对基因表达的调控. 见: 孙大业主编. 细胞信号转导[M]. 第三版, 北京: 科学出版社, 2001. 136-57.

[2] Ong SE, Pandey A. An evaluation of the use of two-dimensional gel electrophoresis in proteomics[J]. Biomol Eng, 2001, 18(5): 195-205.

[3] Hobbs JS, Simonian MH, Betgovargez E, *et al*. Tool for proteomics: An automated approach to protein fractionation[J]. Mod Drug Discovery, 2004, 60-2.

[4] Kachman MT, Wang HX, Schwartz DR, *et al*. A 2-D liquid separations/mass mapping method for interlysate comparison of ovarian cancers[J]. Anal Chem, 2002, 74(8): 1779-91.

[5] Yan F, Subramanian B, Nakeff A, *et al*. A comparison of drug-treated and untreated HCT-116 human colon adenocarcinoma cells using a 2-D liquid separation mapping method based upon chromatofocusing pI fractionation[J]. Anal Chem, 2003, 75(10): 2299-308.

[6] Zheng S, Schneider KA, Barder JT, *et al*. Two-dimensional liquid chromatography protein expression mapping for differential proteomic analysis of normal and O157:H7 *Escherichia coli* [J]. Biotechniques. 2003, 35(6): 1202-12.

[7] Yan F, Sreekumar A, Laxman B, *et al*. Protein microarrays using liquid phase fractionation of cell lysates [J]. Proteomics, 2003, 3: 1228-35.

[8] Lubman DM, Kachman MT, Wang H, *et al*. Two-dimensional liquid separations-mass mapping of proteins from human cancer cell lysates[J]. J Chromat, 2002, 782: 183-96.

[9] 彭 艳, 郑 颖, 蒋 平, 等. 肿瘤细胞蛋白质组的二维液相色谱分离法[J]. 第二军医大学学报, 2004, 25(8): 862-4.