

doi: 10.13241/j.cnki.pmb.2019.09.004

高迁移率族蛋白 1 拮抗剂 BoxA 对血管性痴呆大鼠急性期 海马区域神经炎症的抑制作用 *

黑 悦 魏礼洲 依西才 周跃飞 玉 石 高大宽 刘卫平[△]

(空军军医大学(第四军医大学)附属西京医院神经外科 陕西 西安 710032)

摘要 目的:探究高迁移率族蛋白 1(HMGB1)拮抗剂 BoxA 脑室立体定向注射对双侧颈总动脉闭塞(2VO)后大鼠海马区域神经炎症(血脑屏障通透性、小胶质细胞的激活以及炎症因子水平等)的抑制作用。**方法:**60 只雄性 Sprague-Dawley(SD)大鼠分为假手术组(即 Sham 组)、PBS 组以及 BoxA 组(各组 n=20),分别在行 2VO(n=40, 正常手术)或假手术(n=20, 仅暴露不结扎)后,立即用 BoxA (10 μ g 在 10 μ L PBS 溶液中, n=20)或者等体积 PBS(n=20)立体定向注射到左侧侧脑室中。造模 3 d 后,用 HMGB1 和 NeuN 免疫荧光双染,western blot 观察 HMGB1 出核现象,伊文思蓝(EB)染色和脑含水量测量评价血脑屏障(BBB)通透性,Iba1 免疫荧光染色观察小胶质细胞活化,定量逆转录聚合酶链反应(RT-PCR)检测炎症细胞因子(白介素 1 β (Interleukin-1 β , IL-1 β)和白介素 6(Interleukin-6, IL-6)和肿瘤坏死因子 α (Tumor necrosis factor- α , TNF- α))的基因表达水平。**结果:**相比 Sham 组,PBS 组海马 CA1 亚区 HMGB1 阳性细胞核(%)以及 HMGB1&NeuN 阳性细胞 /HMGB1 阳性细胞(%)显著下降(P<0.01),而 BoxA 组以上改变较 PBS 组部分减少(P<0.05)。PBS 组 EB 渗漏、脑含水量均较 Sham 组显著增加(P<0.001),而 BoxA 组以上指标均较 PBS 组明显减轻(P<0.05)。PBS 组 Iba1 阳性细胞相比 Sham 组明显增多(P<0.01),且炎症因子(IL-1 β 、IL-6 和 TNF- α)表达显著增高(P<0.05);而相比 PBS 组,BoxA 组 Iba1 阳性细胞表达减少(P<0.05),且炎症因子(IL-1 β 、IL-6 和 TNF- α)表达明显减少(P<0.05)。**结论:**HMGB1 抑制剂 BoxA 立体定向注射能够有效缓解急性期血管性痴呆大鼠海马区域的神经炎症反应。

关键词:血管性痴呆;双侧颈总动脉结扎;BoxA;高迁移率族蛋白 1;小胶质细胞

中图分类号:R-33;R743;Q593.2;R338 **文献标识码:**A **文章编号:**1673-6273(2019)09-1618-06

Anti-neuroinflammatory Role of HMGB1 Inhibitor BoxA in the Hippocampal Area of a Rat Model of Vascular Dementia in the Acute Phase*

HEI Yue, WEI Li-zhou, YI Xi-cai, ZHOU Yue-fei, YU Shi, GAO Da-kuan, LIU Wei-ping[△]

(Department of Neurosurgery, Xijing Hospital, Air Force Military Medical University (Fourth Military Medical University),

Xi'an, Shaanxi, 710032, China)

ABSTRACT Objective: To investigate the anti-neuroinflammatory role of stereotactic injection with high mobility group box-1 (HMGB1) inhibitor BoxA in the hippocampal area of rats after bilateral common carotid artery occlusion (2VO). **Methods:** 60 male Sprague-Dawley (SD) rats were divided into the sham group, PBS group and BoxA group (n=20 in each group). 2VO (n=40) or sham operation (n=20, only separation) were performed and BoxA (10 μ g in 10 μ L PBS) or equal amount of PBS was intraventricularly (to the left one) administered immediately after 2VO surgery. Three days later, HMGB1&NeuN immunostaining and western blot were used to observe the HMGB1 translocation, Evans blue (EB) dyeing and brain water content were used to measure the blood-brain barrier (BBB) permeability, Iba1 immunostaining was used to observe microglial activation and reverse transcription-polymerase chain reaction (RT-PCR) was used to assess the levels of pro-inflammatory cytokines (Interleukin-1 β (IL-1 β), Interleukin-6(IL-6) and Tumor necrosis factor- α (TNF- α)). **Results:** Compared with the sham group, there was a significant decrease in HMGB1 positive nucleus (%) and HMGB1&NeuN positive cells/HMGB1 positive cells (%) in the hippocampal CA1 subarea in PBS group (P<0.01). However, compared with the PBS group, the above changes were partially reduced in the BoxA group (P<0.05). In addition, compared with the sham group, PBS group showed significantly increased EB leakage (P<0.001) and increased brain water content (P<0.01). Compared with the PBS group, these changes in the BoxA group were partially adjusted again (P<0.05). Finally, compared with the sham group, the number of Iba1-positive cells in the PBS group increased (P<0.01) and the expression of pro-inflammatory factors (IL-1 β , IL-6 and TNF- α) increased (P<0.05). Compared with the PBS group, the expression of Iba1 positive cells in BoxA group was decreased (P<0.05), and the expression of proinflammatory factors (IL-1 β , IL-6 and TNF- α) were all obviously decreased (P<0.05). **Conclusions:** BoxA suppresses hippocampal neuroinflammatory responses in the rats with 2VO in the acute phase.

Key words: Vascular dementia; Bilateral common carotid occlusion; BoxA; High mobility group box-1; Microglia

* 基金项目:国家自然科学基金重大仪器项目(81627806)

作者简介:黑悦,硕士研究生,主要研究方向:干细胞移植和神经再生, E-mail: heiyue@fmmu.edu.cn

[△] 通讯作者:刘卫平,电话:(029)84774339, E-mail: liuwp@fmmu.edu.cn

(收稿日期:2018-11-30 接受日期:2018-12-25)

Chinese Library Classification(CLC): R-33; R743; Q593.2; R338 Document Code: A

Article ID: 1673-6273(2019)09-1618-06

前言

永久性的双侧颈总动脉结扎 (bilateral common carotid artery occlusion, 2VO) 被广泛用于建立血管性痴呆 (Vascular dementia, VaD) 的动物模型^[1,2], 该模型根据脑血流量的变化和代谢状态可分为三个连续的阶段: 急性期(术后 2-3 天)、慢性阶段(最多 3 个月)和恢复阶段^[3]。近来有研究表明 2VO 大鼠海马 CA1 分区的神经炎症反应造成的二次神经元死亡可能是认知损害发生发展的关键因素^[4-6], 表现为 2VO 模型急性期血脑屏障(blood-brain barrier, BBB)功能障碍和神经胶质细胞的激活(在 VaD 模型中尤以小胶质细胞激活为主)^[7,8]。然而目前关于 VaD 大鼠模型中神经炎症反应的具体干预措施尚未明晰。高迁移率族蛋白 1(High mobility group box-1, HMGB1)在 VaD 急性期能够释放到细胞外基质起到促炎作用^[9-12], 而抑制该分子能否在 VaD 模型中起到积极作用尚不明确。

本研究拟在建立大鼠 2VO 模型的基础上, 采用 HMGB1 特异性小分子拮抗剂 BoxA 脑室立体定向注射干预 HMGB1 的作用, 再用 HMGB1&NeuN 免疫荧光双染和 western blot 观察 HMGB1 核易位现象, 伊文思蓝(EB)染色和脑含水量测量评价 BBB 通透性, Iba1 免疫荧光染色观察小胶质细胞活化, 定量逆转录聚合酶链反应 (reverse transcription-polymerase chain reaction, RT-PCR) 检测炎症细胞因子基因表达水平, 旨在评价 HMGB1 抑制剂 BoxA 对 2VO 大鼠海马区域神经炎症的抑制作用。

1 材料与方法

1.1 实验动物和分组

60 只 4-6 周龄雄性 Sprague-Dawley (SD) 大鼠均来自第四军医大学实验动物中心, 动物实验方案得到西京医院伦理批准并符合美国国立卫生研究院的指南。除了 20 只假手术组(Sham 组, 仅注射无菌磷酸盐(phosphate-buffered saline, PBS, pH 值 7.4)), 其他 80 只大鼠均进行了永久性的双侧颈总动脉结扎 (2VO) 建立血管性痴呆 (VaD) 模型, 被随机分为 BoxA 组(n=20, 脑室立体定向注射溶解于 PBS 中的 BoxA), PBS 组(n=20, 注射 PBS)。实验过程中若有死亡则另行补充。之后各实验中, 各组大鼠数目均为 5 只。

1.2 方法

1.2.1 血管性痴呆大鼠模型以及 BoxA 干预 大鼠麻醉使用水合氯醛(10%, 3ml/kg, i.p.)。在手术中, 首先在颈椎区域的腹侧做一个 1.5 cm 的中线切口。分离脂肪组织及双侧神经纤维后, 显露双侧颈总动脉, 用丝线固定。Sham 组暴露动脉, 未结扎。在整个操作过程中, 温度保持在 37.5℃降低死亡率。总共 10 μg BoxA (HMGBiotech、意大利) 稀释于 10 μL PBS 溶液中, 并使用 15 μL 脑室汉密尔顿显微注射器进行立体定向注射于大鼠左侧侧脑室(AP: 1.5 mm; ML: 1.0 mm; DV: 4.5 mm)。

1.2.2 免疫荧光染色 BoxA 组用 10%水合氯醛(3 mL/kg, i.p.) 麻醉后 4%多聚甲醛灌注。取脑组织准备成 2 μm 厚度。2VO 术

后 3 天进行海马 CA1 亚区 HMGB1 和 NeuN 双染色, 及 Iba1 阳性细胞观察。兔抗 Iba1 (1:100, Abcam, 美国), 鼠抗 NeuN (1:100, Millipore, 美国), 和兔抗 HMGB1 (1:100, Abcam, 美国) 添加后, 4℃孵化过夜。然后用荧光二抗体检测(山羊抗兔 488 和山羊抗鼠 594, 美国)(表达载体、钙、美国)在室温下 4 h。之后, 4 0.0001%, 6-diamidino-2-phenylindole (DAPI) 染色 (Beyotime、上海) 被用来染色细胞核在黑屋子里 10 分钟。HMGB1&NeuN 双染的细胞计数方法: 在海马 CA1 清楚地看到绿色和红色通道共染并与 DAPI 染色后进行细胞计数, 同时观察 10 个以上视野(200×)。肥大体和增厚为活化后的小胶质细胞形态改变。使用共聚焦激光扫描显微镜(FV1000, JPN)获取图像; 使用 image pro plus 6.0(放大 200×)统计。

1.2.3 血脑屏障(BBB)通透性及脑含水量评估 两组大鼠术后 3 d 进行伊文思蓝(Evans Blue, EB)染色和脑含水量分析。将 2 mg/kg EB (Sigma-Aldrich, 美国) 经尾静脉溶解于 0.9% PBS 中, 注入大鼠体内, 让其循环 2 h, 然后将大鼠灌注生理盐水, 将大鼠的大脑在 1.5 mL 甲酰胺中称量均匀, 再孵育 48 小时。根据 620 nm 处的吸光度测定 EB 的渗出量。测量脑含水量时, 大鼠大脑解剖后的重量为脑含水量(湿重), 然后在 100℃干燥 24 小时称量后为干重。最后的数据提出了的价值 (湿重—干重)/湿重×100%。

1.2.4 定量逆转录聚合酶链反应 (RT-PCR) 采用大鼠脑双侧海马。然后检测细胞因子的 mRNA 水平。用 TRIzol 试剂(Invitrogen, 美国) 来分离总 RNA。实验过程中使用的引物如下: IL-1β (5'-CCTTGTGCAAGTGTCTGAAG-3' 和 5'-GGGCTTGGAAGCAATCCTTA-3'), IL-6(5'-AGAAAAGAGTTGTGCAATGGCA-3' 和 5'-GGCAAATTTCTGGTTATATCC-3') 和 TNF-α (5'-CAAGGAGGAGAAGTTCCCA-3' 和 5'-TTGGTGGTTGTCTACGACG-3'), GAPDH (5'-TCCTCATGGACTGATTATGGACA-3' 和 5'-TAATCCAGCAGGTCAGCAAAGA-3')。逆转录后进行使用 1 μg 总 RNA 和 iScript 选择互补脱氧核糖核酸合成的装备(法国)总量的 20 μL 制造商的指示后, 和产品储存在 -20℃。定量 PCR 一步之后, 开始用聚合酶链反应混合物(20 μL)包含 1 μL 每个引物 2 μL 纯化 cDNA 模板。45℃的循环程序包括 5 分钟和 95℃10 年代, 紧随其后的是 35 周期 PCR 在 95℃的 5 s 和 60℃30 年代。将 GAPDH 作为内控, 对靶基因的表达水平进行归一化。

1.2.5 Western blot 将大鼠双侧海马在冰上从大脑中仔细解剖, 用 RIPA 裂解缓冲液匀浆消化。浓度评估后, 用 10% SDS-PAGE 凝胶分离蛋白, 转移到硝化纤维素膜上(Invitrogen, 美国)。5%脱脂牛奶中室温封闭膜 2 小时。使用兔抗 HMGB1(1:1000, Abcam, 美国) 在 4℃过夜。而后 HRP- 结合的二体(1:20000)孵化 1 h。利用 Western Bright ECL 溶液对条带进行可视化, 使用 Gel-Pro Analyzer 6.0 软件(美国)进行分析。

1.3 统计学分析

采用 SPSS 19.0.0 对数据进行统计分析。所有的数据都表示为均数±标准误(Mean±SEM)。多个组比较使用单因素方

差分析,而后使用 Bonferroni 测试组间差别。以 $P<0.05$ 为差异有统计学意义。

2 结果

2.1 BoxA 可抑制血管性痴呆大鼠急性期海马区 HMGB1 的转位

如图 1 和图 2 所示,2VO 模型第 3 天, HMGB1 可从受损

细胞中释放, 导致脑内 HMGB1 水平下降, PBS 组的 HMGB1 阳性细胞核(%)及 HMGB1&NeuN 阳性细胞 /HMGB1 阳性细胞(%)均较 Sham 组下降($P<0.01$)。以上结果表明 HMGB1 可以从神经元中释放。此外, PBS 组海马 HMGB1 表达较 Sham 组明显下降($P<0.05$)。而与 PBS 组相比, BoxA 治疗可以显著逆转海马的这些变化($P<0.05$)。

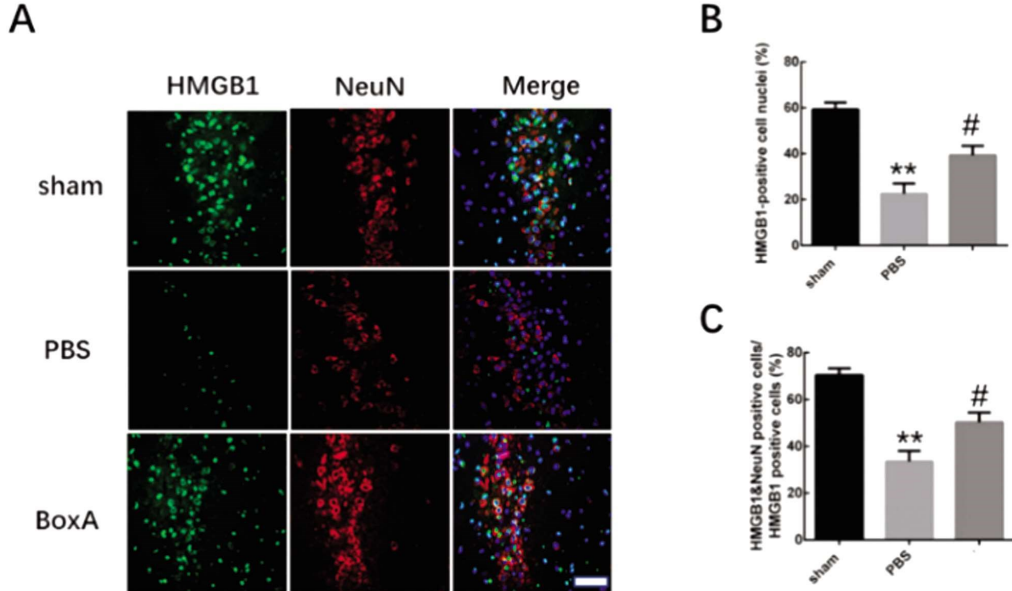


图 1 免疫荧光分析血管性痴呆大鼠急性期海马 CA1 区神经元中 HMGB1 转位

Fig.1 The hippocampal CA1 neuronal HMGB1 translocation in the acute phase in rat model of vascular dementia assessed by immunofluorescence. (A) The HMGB1 & NeuN immunostaining in the hippocampal CA1 area among the three groups; (B) the HMGB1&NeuN positive cells / HMGB1 positive cells(%); (C) HMGB1 positive nucleus (%). Horizontal bar=50 μ m. The data are expressed as mean $\pm$ SEM. ** $P<0.01$ vs. sham group; # $P<0.05$ vs. PBS group

2.2 BoxA 可降低血管性痴呆大鼠急性期血脑屏障通透性

如图 3 所示,2VO 造模 3 d 后, PBS 组 EB 渗漏及脑含水量较 Sham 组增加($P<0.01$), 而与 PBS 组相比, BoxA 组的这些变化被部分逆转($P<0.05$)。

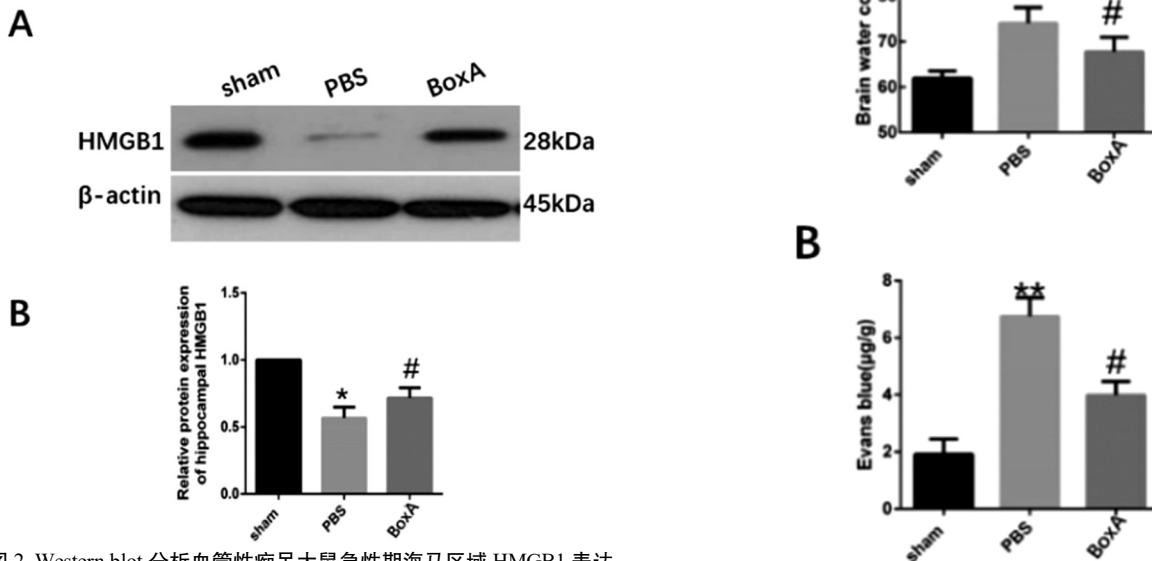


图 2 Western blot 分析血管性痴呆大鼠急性期海马区域 HMGB1 表达
Fig.2 The hippocampal neuronal HMGB1 translocation in the acute phase in rat model of vascular dementia assessed by western blot. (A) The relative protein expression of HMGB1 in hippocampal CA1 area among the three groups; (B) the quantification of western blot. Data are expressed as mean $\pm$ SEM. * $P<0.05$ vs. sham group; # $P<0.05$ vs. PBS group

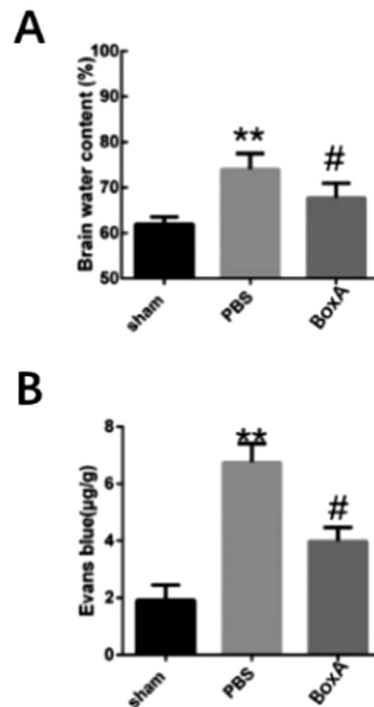


图 3 血管性痴呆大鼠急性期血脑屏障通透性分析

Fig.3 The Blood-brain barrier permeability assessment in rat model of vascular dementia. (A) The brain water content among the three groups; (B) Evans blue (EB) leakage among the three groups. Data are expressed as mean $\pm$ SEM. ** $P<0.01$ sham group; # $P<0.05$ vs. PBS group

2.3 BoxA 可抑制血管性痴呆大鼠急性期海马区域小胶质细胞活化并降低促炎细胞因子表达

如图 4 所示,相比 Sham 组,急性期海马区域 PBS 组小胶质细胞活化明显,Iba1 阳性细胞增多($P<0.01$);与 PBS 组比较,

BoxA 组 Iba1 阳性细胞数量减少($P<0.05$)。另外,术后 3d,相比 PBS 组,BoxA 组 IL-1 β 、IL-6 和 TNF- α mRNA 表达水平较 PBS 组明显降低($P<0.05$)。

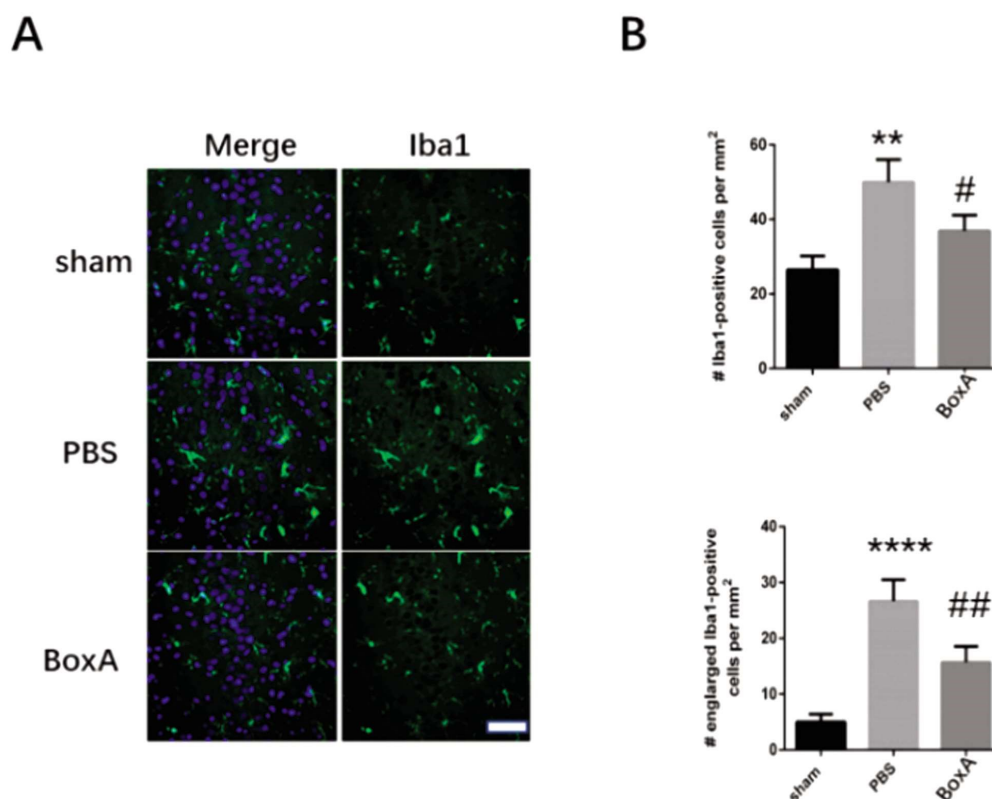


图 4 免疫荧光分析血管性痴呆大鼠急性期海马 CA1 区小胶质细胞激活情况

Fig.4 The hippocampal CA1 microglial activation in the acute phase in rat model of vascular dementia assessed by immunofluorescence

(A) The Iba1 immunostaining in the hippocampal CA1 area among the three groups; (B) the cell counting of Iba1-positive cells (per mm^2) and enlarged Iba1-positive cells (per mm^2). Horizontal bar=50 μm . The data are expressed as mean $\pm$ SEM. ** $P<0.01$, **** $P<0.0001$ vs. sham group;

$P<0.05$, ## $P<0.01$ vs. PBS group.

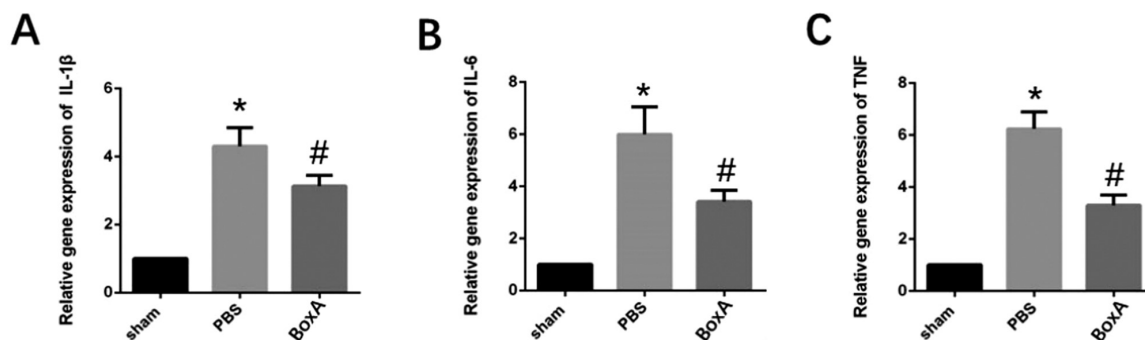


图 5 RT-PCR 分析血管性痴呆大鼠海马区域炎症因子的基因表达情况

Fig.5 The gene expression of pro-inflammatory cytokines of the hippocampus in the acute phase in rat model of vascular dementia assessed by RT-PCR

(A) The gene expression of IL-1 β ; (B) the gene expression of IL-6; (C) the gene expression of TNF- α . Data are expressed as mean $\pm$ SEM.

* $P<0.05$ vs. sham group; # $P<0.05$ vs. PBS group.

3 讨论

在急性损伤或者炎性环境之中,神经元胞内的高迁移率族蛋白 1 (High mobility group box-1, HMGB1)一旦释放到细胞外后作为一种炎症因子能够介导神经炎症反应,而抑制 HMGB1 信号已被报道在神经退行性疾病中具有神经保护作用^[13,14]。既

往研究已证实 HMGB1 在神经炎症和氧化应激中的关键作用,并已使用 BoxA 试剂治疗神经退行性疾病^[15-17]。但迄今为止,BoxA 对 2VO 大鼠的神经炎症抑制作用尚不完全明确。

慢性脑低灌注(chronic cerebral hypoperfusion, CCH)导致持续的脑血流(cerebral blood flow, CBF)减少和认知障碍,最终导致如血管性痴呆 (vascular dementia, VaD) 等神经退行性疾病

[18,19],主要病理生理改变包括白质病变、海马神经元丢失、神经炎症、神经胶质激活等等^[20]。急性期由于血脑屏障(BBB)的破坏和胶质细胞的激活,一方面造成外源性免疫原性物质浸润,一方面释放出炎症因子如 IL-1 β 、IL-6 和 TNF- α ^[21,22]。有研究表明这些炎症因子的调控能够改善 2VO 大鼠模型的整体行为学^[23,24]。本研究中,在 VaD 后,急性期海马区域神经炎症反应明显,能够在 3d 之内出现胶质细胞激活以及血脑屏障破坏等反应,一定程度上验证了如上发现。

HMGB1 是一种已知的损伤相关分子模式(damage-associated molecular patterns,DAMPs)的非组蛋白 DNA 结合蛋白^[25,26],可通过脑感染时的主动分泌或者脑损伤时的被动释放等模式易位到细胞外空间后发挥促炎作用^[27,28]。虽然 HMGB1 可以从先天免疫细胞中释放,但既往研究表明脑缺血后神经元大量释放 HMGB1,导致毗邻神经元的二次损伤^[29,30]。此外,重组 HMGB1 可单独诱导急性炎症,而 BoxA 可抑制炎症反应和氧化应激,导致炎症条件下的行为学和生化结果改善^[28]。而我们也发现在 VaD 模型急性期 HMGB1 在神经元中的表达有易位情况,说明其在海马区域表达降低,可能游离到细胞外间隙或者脑组织之外,下一步将对血液中 HMGB1 的含量进行进一步测定。

HMGB1 的动员模式已在脑缺血、外伤性脑损伤、癫痫和神经退行性疾病的实验模型中得到证实^[31,32]。特别是在缺血条件下,HMGB1 易位表现出越来越多的时间依赖性激活,脑 HMGB1 的表达在术后 1-5 d 显著下调^[33]。在本研究中,我们发现海马区 HMGB1 在术后 3 d 时发生易位。同时,我们也研究了海马 CA1 神经元中 HMGB1 的易位。根据细胞死亡的机制,HMGB1 在神经元中的反应是不同的,只有坏死的细胞才能释放 HMGB1,而不是凋亡的细胞^[34]。由于海马中的坏死细胞死亡在 2VO 模型的急性期优于凋亡细胞在海马 CA1 神经元中可以观察到显著的 HMGB1 释放,这在我们的研究中得到进一步证实。这些结果表明在 CCH 的急性期,海马中 HMGB1 相关机制被激活,海马 CA1 神经元可能是 HMGB1 分泌的细胞基础。

综上所述,HMGB1 激活后作为介导神经炎症的重要分子,在 VaD 大鼠模型急性期在海马区域神经元中呈现出核的动态变化。HMGB1 特异性拮抗剂 BoxA 能够通过抑制 HMGB1 核转位对急性期的海马区域胶质细胞激活以及炎症因子表达水平均有不同程度的抑制作用。

参考文献(References)

- [1] Du S, Wang X, Xiao L, et al. Molecular Mechanisms of Vascular Dementia: What Can Be Learned from Animal Models of Chronic Cerebral Hypoperfusion [J]. *Molecular Neurobiology*, 2017, 54 (5): 3670-3682
- [2] Gupta S, Singh P, Sharma B M, et al. Neuroprotective Effects of Agomelatine and Vinpocetine Against Chronic Cerebral Hypoperfusion Induced Vascular Dementia[J]. *Curr Neurovasc Res*, 2015, 12(3): 240-252
- [3] Farkas E, Luiten P G M, Bari F. Permanent, bilateral common carotid artery occlusion in the rat: A model for chronic cerebral hypoperfusion-related neurodegenerative diseases [J]. *Brain Research Reviews*, 2007, 54(1): 162-180
- [4] D'Haeseleer M, Hostenbach S, Peeters I, et al. Cerebral hypoperfusion: a new pathophysiologic concept in multiple sclerosis [J]. *J Cereb Blood Flow Metab*, 2015, 35(9): 1406-1410
- [5] Sakr H F, Khalil K I, Hussein A M, et al. Effect of dehydroepiandrosterone (DHEA) on memory and brain derived neurotrophic factor (BDNF) in a rat model of vascular dementia [J]. *J Physiol Pharmacol*, 2014, 65(1): 41-53
- [6] Xi Y, Wang M, Zhang W, et al. Neuronal damage, central cholinergic dysfunction and oxidative damage correlate with cognitive deficits in rats with chronic cerebral hypoperfusion[J]. *Neurobiology of Learning and Memory*, 2014, 109: 7-19
- [7] Lee K M, Bang J, Kim B Y, et al. Fructus mume alleviates chronic cerebral hypoperfusion-induced white matter and hippocampal damage via inhibition of inflammation and downregulation of TLR4 and p38 MAPK signaling[J]. *BMC Complement Altern Med*, 2015, 15: 125
- [8] Tian X S, Guo X J, Ruan Z, et al. Long-term vision and non-vision dominant behavioral deficits in the 2-VO rats are accompanied by time and regional glial activation in the white matter [J]. *PLoS One*, 2014, 9(6): e101120
- [9] Edrissi H, Schock S C, Cadonic R, et al. Cilostazol reduces blood brain barrier dysfunction, white matter lesion formation and motor deficits following chronic cerebral hypoperfusion [J]. *Brain Research*, 2016, 1646: 494-503
- [10] Tachibana M, Ago T, Wakisaka Y, et al. Early Reperfusion After Brain Ischemia Has Beneficial Effects Beyond Rescuing Neurons[J]. *Stroke*, 2017, 48(8): 2222-2230
- [11] Ma J, Bo S H, Lu X T, et al. Protective effects of carnosine on white matter damage induced by chronic cerebral hypoperfusion [J]. *Neural Regen Res*, 2016, 11(9): 1438-1444
- [12] Rovere-Querini P, Capobianco A, Scaffidi P, et al. HMGB1 is an endogenous immune adjuvant released by necrotic cells[J]. *EMBO Rep*, 2004, 5(8): 825-830
- [13] Iori V, Maroso M, Rizzi M, et al. Receptor for Advanced Glycation Endproducts is upregulated in temporal lobe epilepsy and contributes to experimental seizures [J]. *Neurobiology of Disease*, 2013, 58: 102-114
- [14] Pisetsky D S. The Translocation of Nuclear Molecules During Inflammation and Cell Death [J]. *Antioxidants & Redox Signaling*, 2014, 20(7): 1117-1125
- [15] Kigerl K A, Lai W, Wallace L M, et al. High mobility group box-1 (HMGB1) is increased in injured mouse spinal cord and can elicit neurotoxic inflammation [J]. *Brain, Behavior, and Immunity*, 2017, 1 (17): 30519-6
- [16] Wang W, Wang B, Lu Q, et al. Inhibition of high-mobility group box 1 improves myocardial fibrosis and dysfunction in diabetic cardiomyopathy[J]. *International Journal of Cardiology*, 2014, 172(1): 202-212
- [17] Yang H, Ochani M, Li J, et al. Reversing established sepsis with antagonists of endogenous high-mobility group box 1 [J]. *Proc Natl Acad Sci U S A*, 2004, 101(1): 296-301
- [18] Murtishaw A S, Heaney C F, Bolton M M, et al. Effect of acute lipopolysaccharide-induced inflammation in intracerebroventricular-streptozotocin injected rats [J]. *Neuropharmacology*, 2016, 101: 110-122

- [19] Yu H, Chen T, Zhou L, et al. Effect of Selective 5-HT₆R Agonist on Expression of 5-HT Receptor and Neurotransmitter in Vascular Dementia Rats[J]. Med Sci Monit, 2017, 23: 818-825
- [20] Iadecola C. The Pathobiology of Vascular Dementia [J]. Neuron, 2013, 80(4): 844-866
- [21] Wu L, Li Y, Yu M, et al. Notch Signaling Regulates Microglial Activation and Inflammatory Reactions in a Rat Model of Temporal Lobe Epilepsy[J]. Neurochem Res, 2018, 43(6): 1269-1282
- [22] Clayton A M, Shao Q, Paaui N D, et al. Postpartum increases in cerebral edema and inflammation in response to placental ischemia during pregnancy[J]. Brain Behav Immun, 2018, 70: 376-389
- [23] Zhang J, Takahashi H K, Liu K, et al. Anti-high mobility group box-1 monoclonal antibody protects the blood-brain barrier from ischemia-induced disruption in rats[J]. Stroke, 2011, 42(5): 1420-1428
- [24] Festoff B W, Sajja R K, van Dreden P, et al. HMGB1 and thrombin mediate the blood-brain barrier dysfunction acting as biomarkers of neuroinflammation and progression to neurodegeneration in Alzheimer's disease[J]. J Neuroinflammation, 2016, 13(1): 194
- [25] Tang D, Kang R, Zeh H J, et al. High-Mobility Group Box 1, Oxidative Stress, and Disease[J]. Antioxidants & Redox Signaling, 2011, 14(7): 1315-1335
- [26] Shi Y, Zhang L, Teng J, et al. HMGB1 mediates microglia activation via the TLR4/NF- κ B pathway in coriaria lactone induced epilepsy [J]. Mol Med Rep, 2018, 17(4): 5125-5131
- [27] Li Z, Li B, Zhu X, et al. Neuroprotective effects of anti-high-mobility group box 1 antibody in juvenile rat hippocampus after kainic acid-induced status epilepticus[J]. Neuroreport, 2013, 24(14): 785-790
- [28] Weber M D, Frank M G, Tracey K J, et al. Stress Induces the Danger-Associated Molecular Pattern HMGB-1 in the Hippocampus of Male Sprague Dawley Rats: A Priming Stimulus of Microglia and the NLRP3 Inflammasome [J]. Journal of Neuroscience, 2015, 35(1): 316-324
- [29] Weber M D, Frank M G, Sobesky J L, et al. Blocking toll-like receptor 2 and 4 signaling during a stressor prevents stress-induced priming of neuroinflammatory responses to a subsequent immune challenge [J]. Brain, Behavior, and Immunity, 2013, 32: 112-121
- [30] Maroso M, Balosso S, Ravizza T, et al. Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures[J]. Nature Medicine, 2010, 16(4): 413-419
- [31] Chen X, Wu S, Chen C, et al. Omega-3 polyunsaturated fatty acid supplementation attenuates microglial-induced inflammation by inhibiting the HMGB1/TLR4/NF- κ B pathway following experimental traumatic brain injury[J]. Journal of Neuroinflammation, 2017, 14(1): 143
- [32] Yang W, Li J, Shang Y, et al. HMGB1-TLR4 axis plays a regulatory role in the pathogenesis of mesial temporal lobe epilepsy in immature rat model and children via the p38MAPK signaling pathway[J]. Neurochemical Research, 2017, 42(4): 1179-1190
- [33] Urbanaviciute V, Furnrohr B G, Weber C, et al. Factors masking HMGB1 in human serum and plasma [J]. J Leukoc Biol, 2007, 81(1): 67-74
- [34] Zhang J, Takahashi H K, Liu K, et al. Anti-high mobility group box-1 monoclonal antibody protects the blood-brain barrier from ischemia-induced disruption in rats[J]. Stroke, 2011, 42(5): 1420-1428

(上接第 1652 页)

- [23] Loera-Arias MJ, Villatoro-Hernández J, Parga-Castillo MA, et al. Secretion of biologically active human interleukin 22 (IL-22) by *Lactococcus lactis*[J]. Biotechnology Letters, 2014, 36(12): 2489-2494
- [24] Wang Bo-Hua, Xu Yang, Li Yan-Ping. Use of the pyrG gene as a food-grade selection marker in *Monascus* [J]. Biotechnology Letters, 2010, 32(11): 1631-1635
- [25] Ng DTW, Sarkar CA. Nisin-inducible secretion of a biologically active single-chain insulin analog by *Lactococcus lactis* NZ9000 [J]. Biotechnology & Bioengineering, 2011, 108(8): 1987-1996
- [26] 王传文, 周宏专, 郭芳芳, 等. 表达空肠弯曲菌 CfrA 蛋白重组乳酸乳球菌的构建及其免疫效果 [J]. 微生物学报, 2016, 56(8): 1326-1334
- [27] Lehmann C, Sieg H, Lampen A, et al. Disturbance of firefly luciferase-based bioassays by different aluminum species[J]. Analytical Biochemistry, 2016, 504: 27-29
- [28] 程佳, 庞田田, 缙晶. 利用荧光素酶报告基因检测维生素 D 生物活性[J]. 药物分析杂志, 2017, 37(07): 1275-1279
- [29] Zhang Fu-quan, Perez-Martin E, Juleff N, et al. A replication-competent foot-and-mouth disease virus expressing a luciferase reporter[J]. Journal of Virological Methods, 2017, 247: 38-44
- [30] Becker JP, Clemens JR, Theile D, et al. Bortezomib and ixazomib protect firefly luciferase from degradation and can flaw respective reporter gene assays[J]. Analytical Biochemistry, 2016, 509: 124-129