.实验研究.

抑制S100B表达改善大鼠重型颅脑损伤后继发性损伤

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【摘要】**目的** 探讨抑制S100B对大鼠重型颅脑损伤(sTBI)后继发性损伤的影响及机制。**方法** 采用液压冲击法建立大鼠sTBI模型,腹腔注射ONO-2506抑制S100B表达,免疫印迹法检测S100B表达表达水平,ELISA检测丙二醛(MDA)、超氧化物歧化酶(SOD)变化,干湿重法检查组织含水量,HE染色分析组织病理变化。**结果** 大鼠血清、脑组织、肺组织S100B蛋白、MDA水平伤后1 h开始上升,伤后6 h达峰值(P<0.05);而SDO水平后1 h开始降低,伤后6 h最低(P<0.05)。抑制S100表达,明显减轻脑组织和肺组织结构损伤,明显降低脑组织和肺组织含水量(P<0.05),明显降低MDA水平(P<0.05),明显增加SOD水平(P<0.05)。**结论** 抑制S100B明显减轻大鼠sTBI脑组织和肺组织损伤,其机制可能与抑制氧化应激反应有关。

【关键词】重型颅脑损伤;S100B蛋白;继发性肺损伤;继发性脑损伤;氧化应激反应;大鼠

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Inhibition of S100B expression improves secondary injury after severe traumatic brain injury in adult rats

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【**Abstract**】**Objective** To investigate the effect of inhibition of S100B expression on secondary injury after severe traumatic brain injury (sTBI) in adults rats. **Methods** The rat sTBI model was established by hydraulic shock method, the expression of S100B was inhibited by intraperitoneal injection of ONO-2506, the expression level of S100B was detected by western blot, the changes of MDA and SOD were detected by ELISA, the tissues water content was detected by dry and wet weight method, and the histopathological changes were analyzed by HE staining. **Results** The levels of S100B expression and MDA in serum, brain tissues and lung tissues of rats began to increase 1 h after injury, and reached a peak at 6 h after injury (P<0.05). The level of SDO began to decrease 1 h after injury and was the lowest at 6 h after injury (P<0.05). Inhibition of S100 expression significantly alleviated structural damage of brain tissues and lung tissues, significantly decreased water content and MDA level of brain tissues and lung tissues (P<0.05), and significantly increased SOD level of brain tissues and lung tissues (P<0.05). **Conclusions** Inhibition of S100B can significantly reduce the secondary damage of brain tissues and lung tissues in adult rats after sTBI, and its mechanism may be related to inhibition of oxidative stress.

【**Key words**】Severe traumatic brain injury; S100B; Secondary lung injury; Secondary brain injury; Oxidative stress; Rats

颅脑损伤(traumatic brain injury,TBI)是一种全球性公共卫生问题,其中重型TBI(severe traumatic brain injury,sTBI)占20%左右,病死率、致残率高[1-4]。sTBI除原发性脑损伤造成较高的致残率和致死率之外,其继发性损伤也严重影响sTBI病人的预后[4~6]。继发性肺损伤是sTBI后常见而严重的并发症,其中急性肺损伤(acute lung injury,ALI)发生率在30%~40%[7,8]。S100B为脑损伤的生物标志物,其血清水平与sTBI严重程度和预后密切相关。本文探讨S100B在sTBI继发性脑损伤和肺损伤中的作用,为临床提供参考。

1 材料与方法

1.1 *实验动物及分组* 108只SPF级SD大鼠,雄性,体重220~250 g,由南方医科大学实验动物中心提供[许可证号:SCXK(粤)2016-0167]。动物饲养及实验遵循《南方医科大学实验动物管理办法(试行)》和《南方医科大学动物实验伦理审查指南(试行)》原则。

①大鼠sTBI后脑组织和肺组织S100B表达变

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化：36只大鼠随机分为6组，即sham组以及伤后5 min、30 min、1 h、6 h、12 h组，每组6只。②抑制S100B表达对大鼠sTBI后脑损伤和肺损伤的影响：72只大鼠随机分为3组，即sham组、sTBI组、抑制剂组，每组24只。

1.2 试剂 BCA蛋白定量试剂盒、组织总蛋白蛋白提取试剂盒购自上海贝博生物公司；CCK-8细胞计数试剂盒、GAPDH抗体购自上海碧云天生物技术有限公司；0.2 μm转印膜购自美国Millipore公司；ONO-2506购自美国GLPBIO公司；ELISA试剂盒购自美国Thermo Scientific公司；S100B抗体购自英国Abcam公司。

1.3 sTBI模型的制作 用13.3%乌拉坦+0.5%氯醛糖配制麻药，按0.65 ml/kg进行腹腔注射麻醉大鼠并固定在立体定向仪上。参考前期实验方法[4,5,7]采用液压冲击法制作模型。沿头部中线切开头皮，分离骨膜后暴露颅骨；右侧前囟后3 mm、矢状线旁开2 mm处用脑科钻行颅骨钻孔，直径4 mm，保持硬膜完整；用胶水连接打击管与骨窗，再用玻璃子水门汀对管周围进行加固，并测试无漏水；待大鼠出现掐尾反射后，液压冲击脑损伤仪对大鼠颅脑造成重型液压冲击，制备模型，峰值冲击压力平均(3.0±0.2)atm，时程平均20 ms。sham组大鼠仅行头皮切开及钻出骨窗，保存硬膜完整，不进行打击。抑制剂组造后后腹腔注射S100B抑制剂ONO-2506(25 mg/kg)，sTBI组注射等量生理盐水；伤后6 h取材。

1.4 HE染色 脑组织、肺组织固定后，石蜡包埋、切片(4 μm)，乙醇梯度脱水后，苏木素染色5 min冲洗后，盐酸乙醇分化30 s冲洗后，置伊红液2 min，常规脱水、透明、封片，显微镜下观察。

1.5 ELISA检测 按ELISA说明书进行操作。将血清按要求进行处理，先加入稀释好后的标准品50 μl、待测样品50 μl，立即加入50 μl生物素标记的抗体。37 ℃温育1 h后洗涤、拍干，重复3次。每孔加入80 μl亲和链酶素-HRP，37 ℃温育30 min后洗涤、拍干，重复3次。每孔加入底物A、B各50 μl，避光37 ℃温育10 min。取出酶标板，迅速加入50 μl终止液。450 nm波长处测定OD值，将样品的OD值代入标准曲线方程式，计算出样品浓度。

1.6 组织含水量测定 将新鲜组织置于洁净的滤纸上称重，记录重量为M0；将组织置于50 ℃烤箱，充分烘干72 h，再称其重量为M1。脑组织含水量=(M0-M1)/M0×100%。肺组织湿/干比=M0/M1。

1.7 免疫印迹法检测S100B蛋白的表达 收集血清、脑组织、肺组织，BCA法定量后，SDS聚丙烯酰胺凝胶电泳，将蛋白转移至硝酸纤维素膜，5% BSA封闭2 h，洗膜后加入S100B一抗(1:1 000)4 ℃过夜，TBST洗3遍后，加入二抗(1:5 000)室温孵育2 h后使用化学发光剂ECL进行反应、曝光。

1.8 统计学方法 采用SPSS 19.0软件分析；计量资料以$\bar{x}\pm s$表示，应用单因素方差分析和LSD-t检验，P<0.05为差异具有统计学意义。

2 结 果

2.1 大鼠伤后血清、脑组织、肺组织S100B蛋白表达变化 大鼠血清、脑组织、肺组织S100B蛋白表达在伤后1 h开始上升，伤后6 h达峰值(P<0.05；图1)。

2.2 抑制剂对大鼠伤后血清、脑组织、肺组织S100B蛋白表达的影响 抑制剂ONO-2506明显抑制大鼠血清、脑组织、肺组织S100B蛋白的表达(P<0.05；图2)。

2.3 抑制S100B表达减轻脑组织和肺组织损伤 sham组神经元结构清晰、排列整齐(图3A)，肺泡结构正常，肺泡腔无渗出(图3D)。sTBI组大鼠脑皮质可见继发性蛛网膜下腔出血、神经元排列紊乱伴水肿(图3B)，且脑水肿程度呈时间依赖性(P<0.05；图4A)，伤后肺泡上皮细胞肿胀、肺泡腔内大量渗出液，毛细血管扩张充血、出血，肺泡结构紊乱(图3E)，且肺水肿程度呈时间依赖性(P<0.05；图4C)。抑制剂组大鼠神经元损伤明显减轻(图3C)，脑水肿明显减轻(P<0.05；图4B)，同时，肺泡腔内渗出明显减少，肺泡结构恢复，毛细血管扩张、充血明显缓解(图3F)，肺水肿程度明显减轻(P<0.05；图4D)。

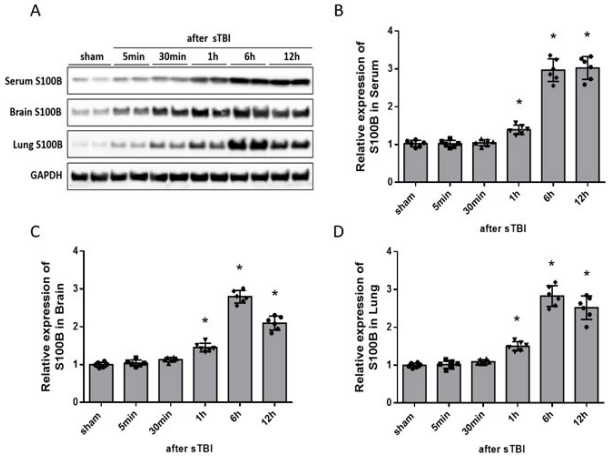

图1 大鼠sTBI后血清、脑组织、肺组织S100B的水平变化 与sham组相比，* P<0.05；A. 蛋白电泳图；B. 血清S100B表达变化；C. 脑组织S100B表达变化；D. 肺组织S100B表达变化

2.4 抑制S100B表达明显抑制血清、脑组织、肺组织氧化应激反应　大鼠伤后血清、脑组织、肺组织MDA水平明显上升，伤后6 h达峰值（P<0.05；图5），而SDO水平明显降低，伤后6 h最低（P<0.05；图5）；抑制S100B表达明显降低大鼠血清、脑组织、肺组织MDA水平（P<0.05；图5），明显增加SOD水平（P<0.05；图5）。

3 讨　论

sTBI可启动一系列病理和生理反应，导致复杂的继发性损伤[9~13]。研究显示，脑源性肺损伤为sTBI病人预后和死亡的独立危险因素[7, 14, 15]。研究表明sTBI继发性损伤的标志性因素有氧化应激损伤、炎症反应、离子稳态失衡等，其中以氧化应激损伤为关键[16~18]。本实验通过液压冲击方法构建大鼠sTBI模型，HE染色结果显示，伤后脑组织和肺组织均出现明显的病理性损伤，血清、脑组织、肺组织MDA水平在伤后5 min开始上升，伤后6 h达峰值，而SDO水平呈相反趋势。这提示sTBI后导致氧化−抗氧化系统失衡，诱导氧化应激损伤参与后续继发性损伤。

S100B是一种钙离子结合蛋白，是S100家族中最具有活性的成员，是脑组织的生物标志物之一[19]。神经细胞受损时，S100蛋白可以经受损的血脑屏障进入外周血，因而，S100蛋白逐渐成为神经系统急性期细胞损伤的标记物，为评估TBI预后的特异性指标；血清S100蛋白水平越高，TBI程度越严重[20~22]。更为重要的是，TBI后脑组织S100B水平增高，不仅引起脑组织本身损伤，还对远隔器官造成损伤[23]，如在蛛网膜下腔出血时，S100B通过与I型肺泡上皮细胞的某些受体结合而导致肺部炎症放大效应，促进神经源性肺水肿的发生[7, 24]。本实验通过发现sTBI大鼠血清、脑组织、肺组织S100B蛋白水平伤后1 h开始增高，伤后6 h达峰值；腹腔注射ONO−2506明显抑制S100B蛋白表达，可以明显减轻伤后脑组织损伤，减轻脑水肿；同时，肺泡腔内渗出明显减少，肺泡结构恢复，肺水肿程度减轻；并且，明显降低MDA水平，而明显增加SOD水平。

总之，抑制S100B明显减轻大鼠sTBI脑组织和

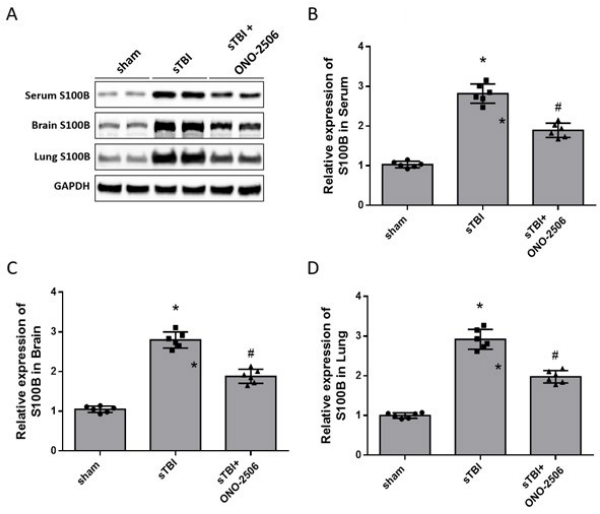

图2 抑制S100B对大鼠sTBI后血清、脑组织、肺组织S100B表达的影响

与sham组比，* P<0.05；与sTBI组比，# P<0.05；A. 蛋白电泳图；B. 血清S100B表达变化；C. 脑组织S100B表达变化；D. 肺组织S100B表达变化

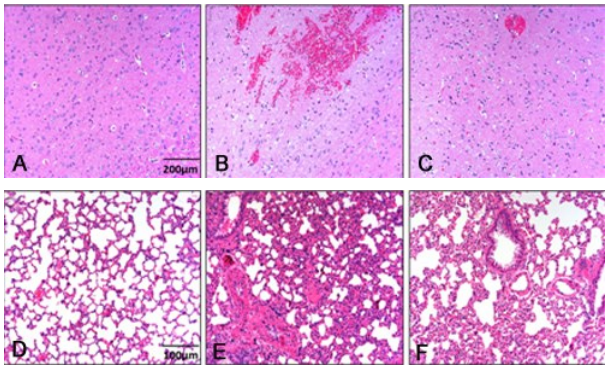

图3 抑制S100B表达对大鼠sTBI后脑组织和肺组织病理损伤的影响

A~C. 大鼠损伤侧皮质脑组织的病理改变情况；D~F. 大鼠伤后肺组织的病理改变情况；A、D. sham组；B、E. sTBI组；C、F. sTBI+ONO−2506组

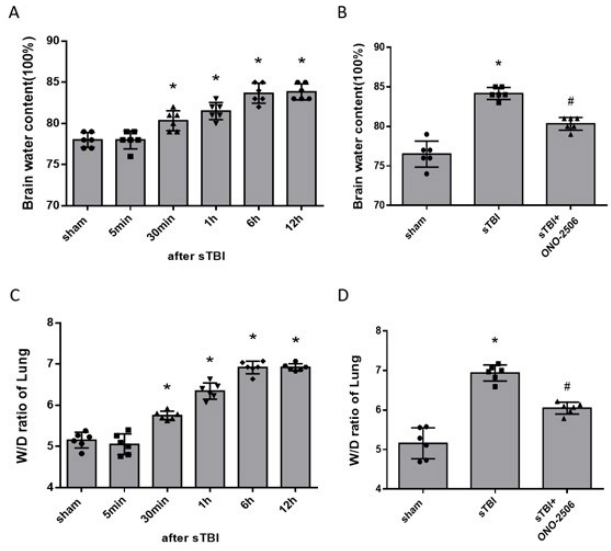

图4 抑制S100B表达减轻大鼠sTBI后脑组织、肺组织水肿

与sham组比，* P<0.05；与sTBI组比，# P<0.05；A. 大鼠sTBI后脑组织含水量变化；B. 抑制S100B表达对大鼠脑组织含水量的影响；C. 大鼠sTBI后肺组织湿/干重比变化；D. 抑制S100B表达对大鼠肺组织湿/干重比的影响

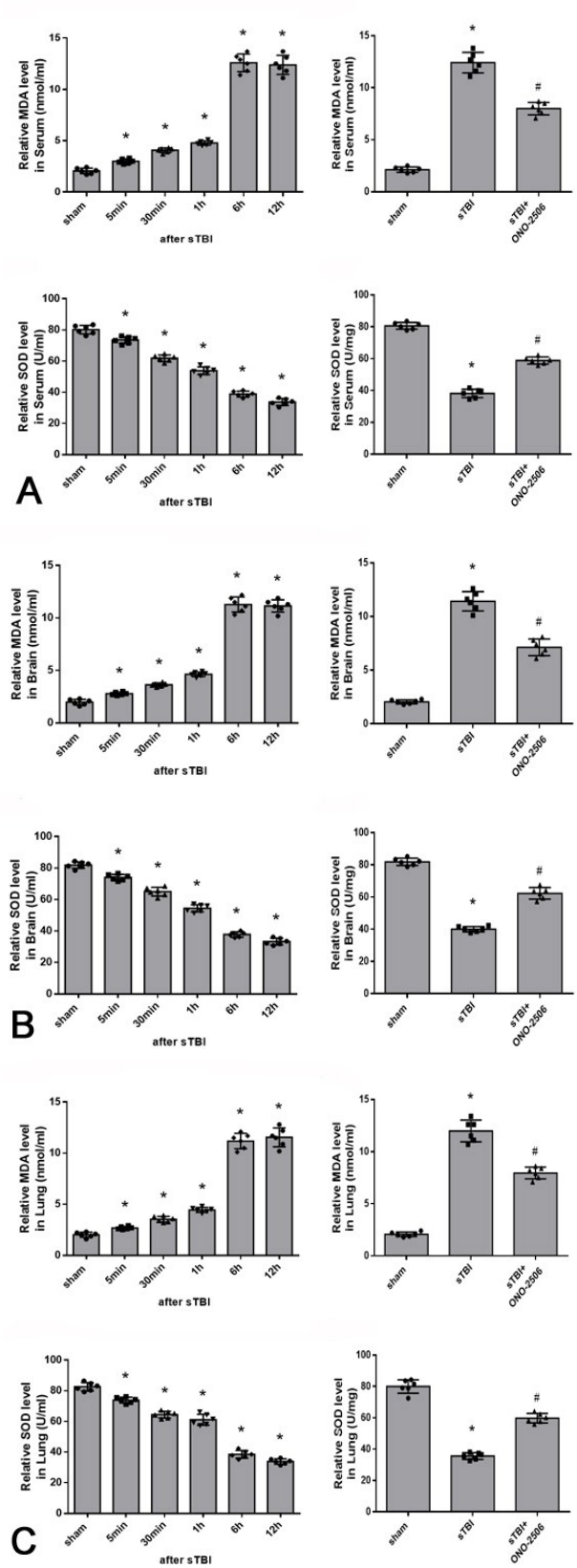

图5 抑制S100B表达对大鼠sTBI后血清、脑组织、肺组织MDA、SOD的影响

与sham组比，* $P<0.05$；与sTBI组比，# $P<0.05$；A. 血清MDA、SOD的变化；B. 脑组织MDA、SOD的变化；C. 肺组织MDA、SOD的变化

肺组织损伤，其机制可能与抑制氧化应激反应有关。

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（上接第507页）

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