

· 基础医学 ·

肌醇依赖酶 1 内切酶活性抑制剂 STF-083010 对肝脏缺血再灌注损伤的保护作用

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[摘要] 目的:探讨肌醇依赖酶 1 (inositol-requiring enzyme 1, IRE1)/剪切型 X-盒结合蛋白 1 (spliced X-box binding protein 1, sXBP1) 通路特异性抑制剂 STF-083010 在小鼠肝脏缺血再灌注损伤中的保护作用及其可能机制。方法:选取健康清洁级雄性 C57BL/6 小鼠 30 只, 随机分成假手术组 (sham 组)、肝缺血再灌注组 (IR 组) 和 STF-083010 预处理+肝缺血再灌注组 (IR+STF-083010 组), 每组 10 只。通过酶联免疫吸附试验 (ELISA 法) 检测小鼠血清丙氨酸氨基转移酶 (alanine aminotransferase, ALT) 和天门冬氨酸氨基转移酶 (aspartate aminotransferase, AST) 水平; HE 染色及 TUNEL 染色检测肝组织损伤情况及肝细胞的凋亡情况; 实时定量 PCR 法检测肝组织中白细胞介素 (interleukin, IL)-6、肿瘤坏死因子 (tumor necrosis factor, TNF)- α 、IL-1 β mRNA 水平; 免疫组织化学法检测肝组织中 sXBP1 蛋白表达水平, 蛋白印迹法检测肝组织中 sXBP1、转录因子 C/EBP 同源蛋白 (C/EBP-homologous protein, CHOP) 蛋白表达水平。结果:与 IR 组相比, IR+STF-083010 组小鼠血清 ALT、AST 水平明显降低 ($P < 0.01$), 组织学上, 肝损伤情况得到明显改善 ($P < 0.01$), 肝组织中 IL-6、TNF- α 、IL-1 β mRNA 水平及 sXBP1、CHOP 蛋白水平明显降低 ($P < 0.01$)。结论:STF-083010 预处理可通过抑制 sXBP1/CHOP 通路减轻肝脏缺血再灌注损伤。

[关键词] STF-083010; 缺血再灌注损伤; 肝脏; 内质网应激

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Protective mechanism of inositol-requiring enzyme 1 endoribonuclease specific inhibitor STF-083010 against liver ischemia-reperfusion injury

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[Abstract] **Objective:** To explore the protective effect of STF-083010, a specific inhibitor of inositol-requiring enzyme 1 endoribonuclease (IRE1)/spliced X-box binding protein 1 (sXBP1) on liver ischemia-reperfusion (IR) injury and the possible mechanisms. **Methods:** Thirty C57BL/6 mice were randomly divided into 3 groups (10 in each group): sham operation group (sham group), hepatic ischemia-reperfusion group (IR group), and STF-083010 pretreatment plus hepatic ischemia-reperfusion group (IR+STF-083010 group). Levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were detected by enzyme-linked immunosorbent assay (ELISA). The histological damage of liver and the apoptosis of hepatocytes were evaluated by hematoxylin-eosin (HE) and TUNEL staining. The expression of interleukin (IL)-6, tumor necrosis factor (TNF)- α , and IL-1 β mRNA were examined by RT-qPCR. sXBP1 and CHOP proteins in liver tissues were determined by immunohistochemistry and Western blot. **Results:** Compared to IR group, plasma ALT and AST levels in STF-083010+IR group were significantly lower ($P < 0.01$). Liver tissues in STF-083010+IR group were more slightly damaged than those in IR group ($P < 0.01$). The mRNA levels of IL-6, TNF- α and IL-1 β in IR+STF-083010 group and the protein levels of sXBP1 and CHOP were significantly lower than those of IR group ($P < 0.01$). **Conclusion:** Pretreatment of STF-083010 can alleviate liver IR injury by inhibiting sXBP1/CHOP signal pathway.

[Key words] STF-083010; ischemia-reperfusion injury; liver; ER stress

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肝脏缺血再灌注(ischemia-reperfusion, IR)损伤是肝部分切除术和肝移植术后常见的并发症,其发病机制复杂。该过程涉及到活性氧的释放,炎症细胞的激活,炎性介质的产生等诸多因素^[1]。近年来的研究发现内质网应激(endoplasmic reticulum stress, ERS)反应能加重炎症反应,是导致肝脏缺血再灌注损伤的重要病理机制之一^[2]。STF-083010是肌醇依赖酶1(inositol-requiring enzyme 1, IRE1)核糖核酸酶特定抑制剂,可特异性阻断IRE1-剪切型X-盒结合蛋白1(spliced X-box binding protein 1, sXBP1)信号通路,有效降低sXBP1的表达^[3]。本研究旨在探讨STF-083010预处理对肝脏缺血再灌注损伤的影响。

1 材料和方法

1.1 材料

30只健康雄性C57BL/6小鼠,6~8周龄,体重15~20g,购自南京医科大学实验动物中心,无特殊病原体(SPF)环境下饲养,自由进食、饮水。动物实验已获得动物伦理委员会的批准。STF-083010(Selleck公司,美国);丙氨酸氨基转移酶(alanine aminotransferase, ALT)和天门冬氨酸氨基转移酶(aspartate aminotransferase, AST)检测试剂盒(北京达科为);TRIzol(南京诺唯赞)、肿瘤坏死因子(tumor necrosis factor, TNF)- α 、白细胞介素(interleukin, IL)-6和IL-1 β 引物(Invitrogen公司,英国);逆转录试剂盒(TaKaRa公司,日本);ChamQ通用型SYBR Green qPCR Master Mix(南京诺唯赞);TUNEL染色试剂盒(武汉赛维尔);RIPA裂解液(上海碧云天);sXBP-1抗体(Proteintech公司,美国)、转录因子C/EBP同源蛋白(C/EBP-homologous protein, CHOP)抗体、GAPDH抗体、兔源性二抗(CST公司,美国);超敏ECL发光液(苏州新赛美)。

1.2 方法

1.2.1 动物模型

将30只小鼠随机分成3组,每组10只。①假手术组(sham组):小鼠仅接受腹腔麻醉和开、关腹操作;②肝缺血再灌注组(IR组):小鼠在建立IR前30min,按30mg/kg腹腔注射生理盐水;③STF-083010预处理+肝缺血再灌注组(IR+STF-083010组):小鼠在建立IR前30min,按30mg/kg腹腔注射STF-083010。

肝脏缺血再灌注模型建立具体步骤:小鼠术前12h禁食,不禁饮,5%水合氯醛腹腔注射,麻醉成功

后,取小鼠腹部正中切口,用生理盐水棉签明确分离肝左、中叶后,使用无损伤血管夹夹闭通往肝左叶和肝中叶的肝蒂,此时可见缺血肝叶色泽改变,关腹。90min后,松开血管夹,缺血肝叶恢复血供,肝脏颜色转红,关腹。6h后处死小鼠,收取静脉血和肝脏组织,部分组织用4%甲醛固定,其余肝脏样本生理盐水冲洗后,液氮保存。

1.2.2 RNA提取和实时定量PCR

采用TRIzol法提取组织和细胞RNA,并测定RNA的浓度及纯度(NanoDrop公司,美国)。首先按照反转录试剂盒说明书进行逆转录,所得cDNA进行实时定量PCR,反应使用SYBR qPCR Master Mix试剂,并在ABI 7900 Fast Real-Time PCR system上进行。按照 $2^{-\Delta\Delta Ct}$ 法来计算相对表达量,实验重复3次。

1.2.3 蛋白提取及Western blot

提取总蛋白时,使用含1%蛋白酶抑制剂和1%磷酸酶抑制剂的RIPA裂解液裂解细胞。测定浓度后,按比例加入5 \times 上样缓冲液,100 $^{\circ}$ C水浴10min。蛋白经SDS-PAGE电泳分离后,将相应分子量的蛋白湿转至PVDF膜上,然后在室温下用快速封闭液封闭10min;分别加入一抗4 $^{\circ}$ C孵育过夜,室温下二抗孵育2h, TBST洗膜后使用超敏ECL曝光液成像分析,实验重复3次。

1.2.4 肝功能检测

取小鼠静脉血,3000r/min离心10min,收集上清。根据试剂盒说明测定血清ALT和AST水平。

1.2.5 肝脏组织病理学检查

取10%福尔马林溶液固定的肝组织,常规切片后,HE染色,于光镜下观察肝小叶结构、肝细胞水肿及坏死的情况。

1.2.6 TUNEL染色观察细胞凋亡情况

根据TUNEL染色试剂盒说明操作,具体步骤为:用免疫染色固定液4%多聚甲醛固定细胞30~60min;PBS洗涤2次,每次10min;加入免疫染色强力通透液室温孵育5min;PBS洗涤2次,每次10min;在样品上加50 μ L TUNEL检测液,37 $^{\circ}$ C避光孵育60min;PBS洗涤3次;用抗荧光淬灭封片液封片后,于荧光显微镜下观察并拍照。

1.3 统计学方法

应用GraphPad7.0软件对实验数据进行统计学处理。所有定量数据以均数 \pm 标准差($\bar{x} \pm s$)表示,多组间差异采用方差分析,组间差异两两比较采用SNK法。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 血清转氨酶水平变化

收取小鼠静脉血,测定各组小鼠血清ALT、AST水平。与sham组相比,IR组小鼠肝酶明显升高($P < 0.01$,图1);与IR组小鼠相比,IR+STF-083010预处理组小鼠血清ALT、AST水平显著降低,差异有统计学意义($P < 0.01$,图1)。

2.2 各组肝脏组织学改变

灌注6 h后,收取小鼠肝脏标本行HE染色。sham组小鼠肝脏组织排列正常,肝小叶结构清晰,肝细胞成条索状排列,无肝细胞坏死;IR组肝细胞排列紊乱,水肿明显,并可见大片肝细胞坏死(图2A);而IR+STF-083010IR组肝细胞呈轻度水肿,肝小叶结构基本完整,坏死肝细胞较IR组也明显减少。同样地,TUNEL染色也发现IR组肝细胞凋亡较sham组显著增加,而STF-083010预处理能明显减少肝细胞凋亡(图2B)。表明STF-083010预处理能有效减轻小鼠肝脏缺血再灌注损伤。

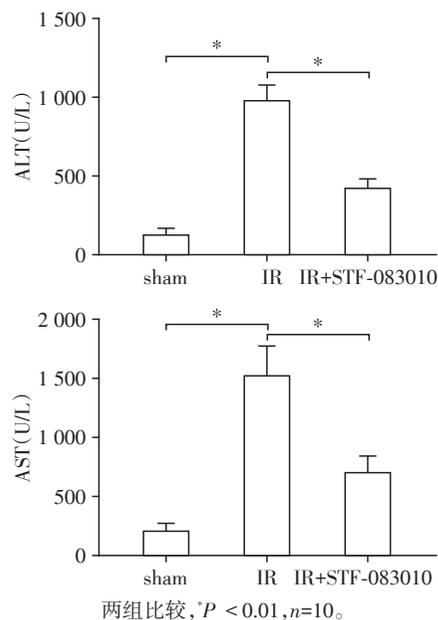
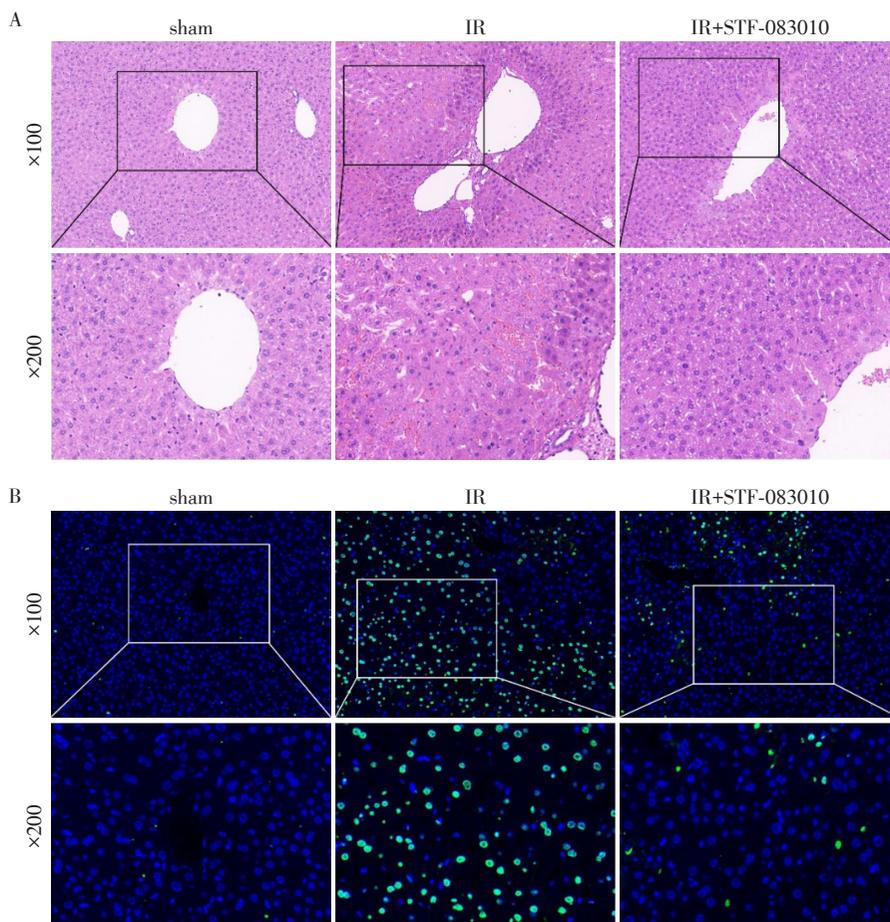


图1 STF-083010预处理对缺血再灌注小鼠ALT和AST的影响

Figure 1 Effects of STF-083010 on ALT and AST in mice with liver ischemia/reperfusion injury



A:再灌注后6h各组肝脏样本的HE染色代表性图片;B:再灌注6h后各组肝脏样本TUNEL染色代表性图片。

图2 STF-083010预处理对肝缺血再灌注损伤小鼠组织病理学的影响

Figure 2 Effects of STF-083010 on histopathology in mice with liver ischemia/reperfusion injury

2.3 各组肝脏IL-6、TNF-α、IL-1β表达量的变化

与 sham 组相比,IR 组小鼠 IL-6、TNF-α、IL-1β mRNA 水平明显升高 ($P < 0.01$, 图 3); IR+STF-083010 组肝脏 IL-6、TNF-α、IL-1β mRNA 的表达水平较缺血再灌注组明显降低,差异有统计学意义 ($P < 0.01$, 图 3)。

2.4 各组肝脏sXBP1蛋白表达水平的变化

免疫组化染色发现 sham 组小鼠肝脏组织中仅有少量的 sXBP1 蛋白表达,相比 sham 组,IR 组中 sXBP1 蛋白的表达明显升高(图 4A);STF-083010 预处理则能显著降低缺血再灌注导致的 sXBP1 和 CHOP 蛋白水平增高(图 4B)。

3 讨论

肝脏缺血再灌注损伤是一个极其复杂的病理

生理过程,缺血过程会引起细胞内酸中毒、钙超载等;再灌注过程会导致活性氧释放、氧化应激、炎症反应等,最终导致肝细胞凋亡和坏死,进而出现肝功能不全,是影响肝移植、肝切除手术预后效果的主要因素之一^[4-5]。因此,探索预防或减轻肝脏缺血再灌注损伤的相关研究,具有重要的临床意义。

近年来的研究表明,ERS 与 IR 损伤密切相关,ERS 可通过激活经典的未折叠蛋白反应(unfolded protein response, UPR)抑制错误蛋白质翻译和/或指导错误蛋白质进行正确折叠修饰,进而减轻细胞应激损伤。在应激早期,细胞能迅速启动生存途径,降低内质网负荷,并维持其稳态。当内质网受到长时间的刺激或刺激强度过大时,内质网内大量错误蛋白或未折叠蛋白超过了细胞生存途径所能处理

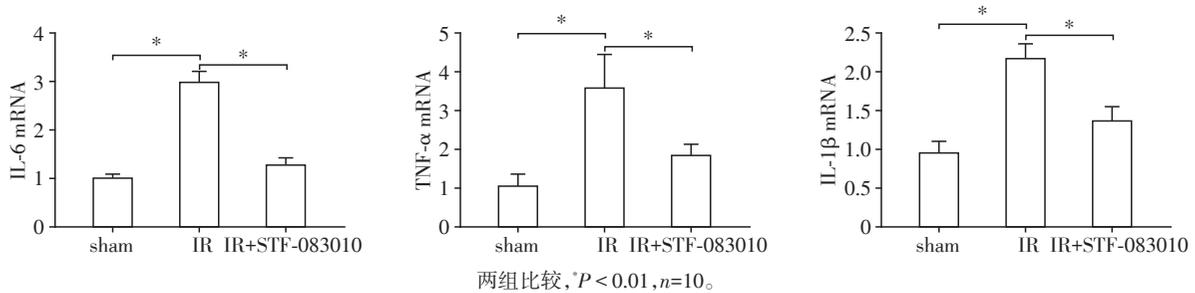
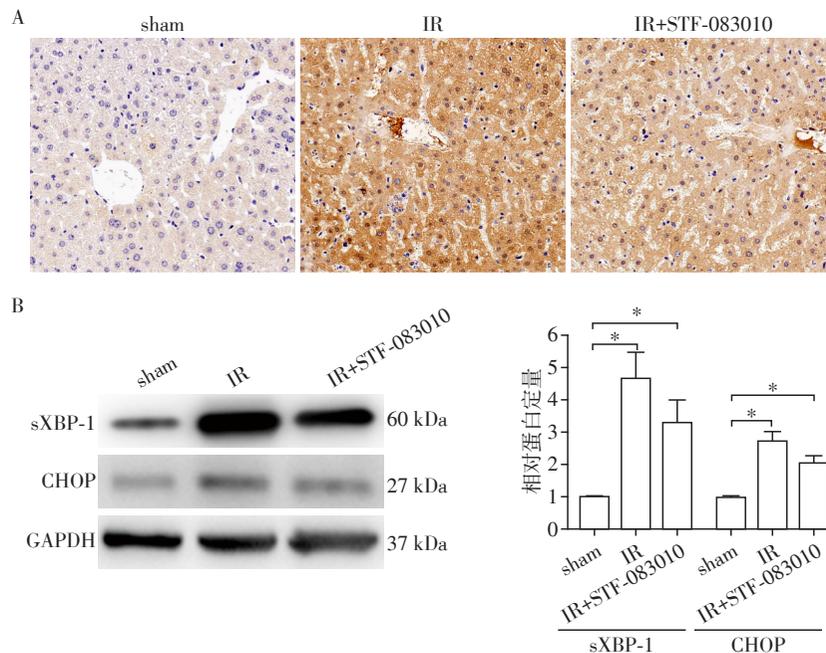


图3 STF-083010 预处理对缺血再灌注小鼠肝脏组织中 IL-6、TNF-α、IL-1β 水平的影响

Figure 3 Effects of STF-083010 on the levels of IL-6, TNF-α, IL-1β in liver with ischemia/reperfusion injury



A: 各组肝脏样本的 sXBP1 蛋白 IHC 染色代表性图片 (×200); B: Western blot 检测各组肝脏样本的 sXBP1、CHOP 蛋白表达, * $P < 0.01$, $n = 10$ 。

图4 STF-083010 预处理对缺血再灌注小鼠肝脏组织中 sXBP1、CHOP 蛋白表达的影响

Figure 4 Effects of STF-083010 on the expression of sXBP1 and CHOP in liver with ischemia/reperfusion injury

的范围,将使内质网内的稳态遭到破坏,引起内质网持续性应激,激活CHOP等介导的细胞凋亡程序,导致细胞凋亡^[6-7]。

经典的ERS有3条信号通路,即蛋白激酶样内质网激酶(PKR-like ER kinase, PERK)/真核翻译起始因子2 α (eukaryotic translation initiation factor-2 α , eIF2 α)、IRE1/sXBP1、转录激活因子6(activating transcription factor 6, ATF6)。其中,IRE1-sXBP1通路是ERS中最为经典和保守的通路^[8]。IRE1在决定细胞在ERS状态下应对各种刺激的转归中扮演重要角色^[9-10]。IRE1磷酸化活化后可激活其具有核酸内切酶活性的RNase结构域,特异性地剪切XBP1 mRNA,促进剪接形式的XBP1蛋白即sXBP1表达。sXBP1是强效转录因子,也是ERS中重要的效应分子,它能进一步促进下游信号分子CHOP等的表达,导致细胞凋亡,加重炎症损伤^[11]。薛强等^[12]研究发现使用短发夹RNA干扰XBP1能有效减轻肝移植时的缺血再灌注损伤。STF-083010是一种新型IRE1-sXBP1通路特异性抑制剂,在不影响IRE1激酶活性的情况下,特异性抑制IRE1的核酸内切酶活性,从而抑制XBP1 mRNA剪接,降低sXBP1蛋白表达^[13]。最近研究显示其在多种肝脏损伤中具有保护作用。Lebeauin等^[14]报道STF-083010可以缓解高脂饮食诱导的小鼠非酒精性脂肪肝病,其机制与NOD样受体家族核苷酸结合寡聚化结构域样受体3(NOD-like receptor family pyrin domain containing 3, NLRP3)炎症小体激活及细胞死亡相关。Chen等^[15]研究显示STF-083010改善四氯化碳诱导的肝损伤,并上调miR-122表达,进而缓解肝脏纤维化。本课题组的前期研究发现STF-083010可以通过阻断IRE1-sXBP1信号通路,激活自噬信号,减轻硫代乙酰胺(thioacetamide, TAA)引起的小鼠肝脏损伤^[16]。徐璐等^[17]的研究发现STF-083010可以减轻大鼠肾脏缺血再灌注损伤,改善肾功能。然而,关于STF-083010在肝脏缺血再灌注中作用的研究尚未见报道,本研究结果显示STF-083010能显著降低小鼠缺血再灌注肝脏组织中sXBP1、CHOP蛋白的表达,抑制ERS相关炎症和凋亡通路,对小鼠肝脏缺血再灌注损伤具有保护作用。

综上所述,本研究显示STF-083010对小鼠肝脏缺血再灌注损伤具有保护作用,其机制可能与抑制ERS相关凋亡通路有关。提示STF-083010有可能在肝脏外科的临床中有其应用价值,有望为肝缺血再灌注损伤的防治提供一个新的干预靶点。

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