

NF- κ B对肠缺血再灌注肝损伤P-选择素表达和中性粒细胞浸润的作用

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■背景资料

肠缺血再灌注损伤(IIR)是严重创伤、休克等疾病治疗过程中经常发生的病理生理过程,其造成的损伤不仅局限于肠道本身,并且含有大量毒素的血液还可引起远隔脏器的严重损伤。早期研究认为许多损伤介质直接参与了IIR肝损伤的发病过程。近期研究表明,这些介质的作用可受转录因子NF- κ B的调节。

研究和探讨NF- κ B在IIR肝损伤中的作用以及NF- κ B抑制剂脯氨酸二硫代氨基甲酸酯(PDTC)预处理对IIR肝损伤P-选择素(P-selectin)的表达和中性粒细胞浸润的影响有较大意义。

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Effect of nuclear factor- κ B on P-selectin expression and neutrophil infiltration in liver injury induced by intestinal ischemia/reperfusion in rats

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Abstract

AIM: To investigate the effect of nuclear factor- κ B (NF- κ B) on P-selectin expression and neutrophil accumulation in liver injury induced by intestinal ischemia/reperfusion (I/R) in rats.

METHODS: Twenty-four Wistar rats were randomly assigned into sham operation (control, $n = 8$), intestinal I/R ($n = 8$), and pyrrolidine di-thiocarbamate (PDTC) treatment ($n = 8$). The rats in I/R and PDTC group received 1 h SMA occlusion and 2 h reperfusion, and those in PDTC group was also intraperitoneally injected with 20 g/L PDTC (100 mg/kg) 1 h before operation.

Liver histology was observed under light microscope. The level of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and interleukin-6 (IL-6), and liver tissue superoxide dismutase (SOD), myeloperoxidase (MPO) contents were measured. The immunohistochemical expression of liver NF- κ B and P-selectin as well as Western blot analysis of liver NF- κ B were assayed.

RESULTS: Liver injury was induced by intestinal I/R, characterized as histological damage of edema, hemorrhage and polymorphonuclear infiltration as well as the significant rise of serum ALT (from 143.16 ± 53.02 to 192.31 ± 42.09 U/L, $P < 0.05$) and AST level (from 387.46 ± 78.74 to 507.56 ± 96.26 U/L, $P < 0.01$). In comparison with that in control group, the level of serum IL-6 increased significantly (from 22.51 ± 6.10 to 42.85 ± 7.35 ng/L, $P < 0.01$) in I/R group, and tissue SOD decreased (from 244.87 ± 25.11 to 173.21 ± 16.60 U/mgprot, $P < 0.01$) while MPO increased (from 2.36 ± 0.56 to 4.32 ± 0.77 U/g, $P < 0.01$) significantly. Strong positive expression of NF- κ B p65 and P-selectin were observed. After PDTC administration, the level of serum IL-6, tissue SOD and MPO were improved markedly (28.08 ± 7.55 ng/L, $P < 0.05$; 253.45 ± 25.21 U/mgprot, $P < 0.01$; 3.58 ± 0.49 U/g, $P < 0.05$) as compared with those in I/R group, and the expression of NF- κ B and P-selectin were weakened markedly.

CONCLUSION: The activation of NF- κ B plays an important role in the pathogenesis of liver injury induced by intestinal through up-regulating the neutrophil infiltration and liver P-selectin expression. PDTC as an inhibitor of NF- κ B can prevent this injury through inhibiting NF- κ B activity.

Key Words: Liver injury; Intestinal ischemia/reperfusion; NF- κ B; P-selectin

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摘要

目的: 探讨核转移因子- κ B(NF- κ B)在肠缺血再灌注肝损伤发病机制中的作用及其对P-选择素(P-selectin)表达和中性粒细胞浸润的影响。

方法: Wistar大鼠24只随机分成对照(Control组)、肠缺血再灌注(I/R组)和脯氨酸二硫代氨基甲酸酯(PDTC)治疗组(PDTC组), 每组8只。I/R和PDTC组大鼠行肠系膜上动脉夹闭1 h再灌注2 h。PDTC组于手术前1 h给予20 g/L PDTC 100 mg/kg ip。观察肝组织病理学及其肝功能变化, 检测血清IL-6, 肝组织匀浆超氧化物歧化酶(SOD)和髓过氧化物酶(MPO)水平。免疫组化法观察肝组织P-selectin和NF- κ B表达并采用Western blot法检测肝NF- κ B的水平。

结果: 肠缺血再灌注诱发了肝损伤, 表现为肝水肿、出血和炎性粒细胞浸润。与对照组相比, I/R组血清ALT、AST、IL-6水平明显升高(143.16 ± 53.02 至 192.31 ± 42.09 U/L, $P < 0.05$; 387.46 ± 78.74 至 507.56 ± 96.26 U/L, $P < 0.01$; 22.51 ± 6.10 至 42.85 ± 7.35 ng/L, $P < 0.01$)。肝组织SOD活性降低、MPO含量明显升高(244.87 ± 25.11 至 173.21 ± 16.60 U/mgprot, $P < 0.01$; 2.36 ± 0.56 至 4.32 ± 0.77 U/g, $P < 0.01$)。肝组织的P-selectin和NF- κ B表达增强。采用PDTC预处理, 与I/R组相比, 肝损伤程度减轻, 血清ALT(128.63 ± 38.94 U/L)、AST(462.86 ± 60.84 U/L)以及IL-6(28.08 ± 7.55 ng/L)水平明显降低($P < 0.01$, $P < 0.05$, $P < 0.05$)。肝脏氧化损伤及白细胞浸润减弱, 表现为肝组织SOD活性升高(253.45 ± 25.21 U/mgprot, $P < 0.01$)、MPO含量降低(3.58 ± 0.49 U/g, $P < 0.05$), 同时伴有肝组织中的P-selectin和NF- κ B表达减弱。

结论: 肠缺血再灌注诱发肝损伤, 伴有明显的中性粒细胞浸润和肝组织P-selectin的表达增强, NF- κ B的活化在此损伤过程中起重要作用。PDTC通过抑制NF- κ B活性对肠缺血再灌注肝损伤起保护作用。

关键词: 肝损伤; 肠缺血再灌注; 核转移因子 κ B; P-选择素

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0 引言

肠缺血再灌注(intestinal ischemia/reperfusion,

IIR)造成的损伤不仅局限于肠道本身, 含有大量毒素的血液还可引起远隔脏器的严重损伤。通常认为, IIR是造成MODS和SIRS的始动因素, 其引发的MODS是临床上常见的并发症和重要的死亡原因^[1-3]。肝脏做为肠缺血再灌注后接受含有毒素血液的最直接器官, 其不仅分解和代谢这些毒素, 同时也受这些毒素的损伤并产生新的炎症因子, 研究肠缺血再灌注肝脏损伤机制及其保护预处理十分必要。

IIR肝损伤的机制较复杂。早期研究认为, 再灌注血液中的毒性氧化物质、细胞炎症因子和化学因子直接作用到靶器官细胞是造成损伤的主要原因, 细胞间黏附因子和中性粒细胞浸润在IIR肝损伤中起重要作用^[4-7]。但新近研究表明, 这些介质是通过基因诱导完成的, 核转录因子 κ B(NF- κ B)可上调这些炎症介质的表达^[8-11]。我们探讨NF- κ B在IIR肝损伤中的作用以及NF- κ B抑制剂脯氨酸二硫代氨基甲酸酯(PDTC)预处理对IIR肝损伤P选择素(P-selectin)的表达和中性粒细胞浸润的影响。

1 材料和方法

1.1 材料 δ Wistar大鼠24只, 体质量200-260 g, 由大连医科大学动物中心提供。随机分为3组($n = 8$): 对照组, 模型组(I/R组), PDTC预处理组。大鼠100 g/L水合氯醛350 mg/kg ip麻醉后, 上腹正中切口入腹, 对照组大鼠剖腹后分离肠系膜上动脉但不结扎; I/R组采用Megison法^[12]制作肠缺血再灌注模型, 分离并结扎肠系膜上动脉1 h再灌注2 h; PDTC预处理组手术操作同I/R组, 术前1 h给予20 g/L PDTC 100 mg/kg ip。对照组和I/R组给予等量生理盐水。再灌注2 h后处死动物, 取血及肝组织。

1.2 方法 肝组织经40 g/L中性甲醛固定后作4 μ m石蜡切片, HE染色, 光镜下观察肝脏组织形态学改变。分离的血清采用OLYMPUS AU1000生化自动分析仪进行ALT, AST活性测定。血清IL-6的检测采用放免试剂盒(解放军总医院科技开发中心放免所)进行检测。肝组织超氧化物歧化酶(SOD)和髓过氧化物酶(MPO)的测定分别采用硫代巴比妥酸法和过氧化氢还原法(试剂盒均为南京建成生物公司产品)。肝组织NF- κ B p65和P-selectin的免疫组化检测采用SP免疫组化法, 5 μ m石蜡切片置二甲苯中脱蜡, 30 mL/L过氧化氢孵育消除内源性过氧化物酶, 正常非免疫动物血清封阻30 min, 分别滴加

■ 研发前沿

近年, 对肠缺血再灌注远隔器官的损伤中NF- κ B的作用研究越来越多。研究表明, 缺血再灌注损伤后的炎症介质和毒素受到NF- κ B的调控, 对其机制的研究是目前关注的热点。

■创新盘点

本文首先针对肠缺血再灌注肝损伤中NF- κ B对P-选择素表达和中性粒细胞浸润进行了研究,发现肠缺血再灌注诱发肝损伤,伴有明显的中性粒细胞浸润和肝组织P-selectin的表达增强,NF- κ B的活化在此损伤过程中起重要作用。

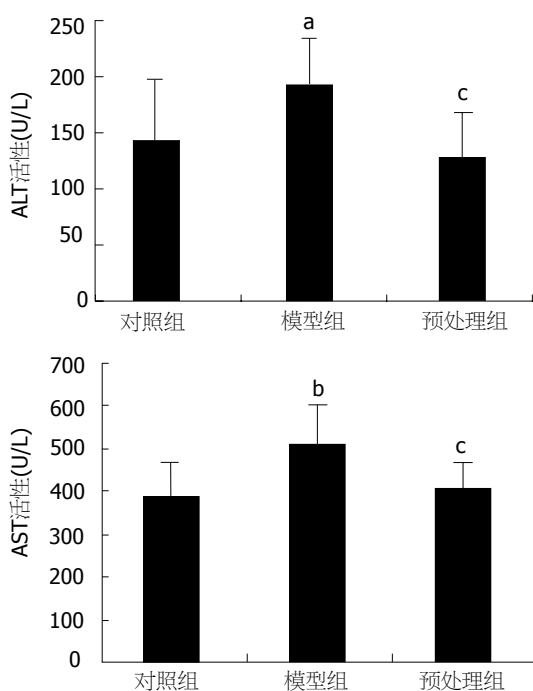


图1 PDTC对肠缺血再灌注大鼠血清ALT、AST水平的影响。模型组与对照组相比,^a $P<0.05$,^b $P<0.01$; PDTC预处理组与模型组相比, $P<0.05$,^d $P<0.01$ 。

多克隆兔抗鼠NF- κ B p65和P-selectin一抗(分别为NeoMarkers和博士德生物工程公司产品)4℃过夜。再加入生物素标记的二抗(羊抗兔IgG抗体)和链霉素抗生物素蛋白。DAB显色,苏木精复染。NF- κ B的Western blot分析,按蛋白提取试剂盒(Pierce, Meridian Road, Rockford)说明书提取肝组织核蛋白,用考马斯亮蓝法(南京建成生物技术公司产品)测定蛋白浓度。蛋白于100 g/L聚丙烯酰胺凝胶电泳分离后转膜,加入NF- κ B p65(NeoMarkers公司产品)兔抗大鼠多克隆抗体(1:1 000稀释)4℃过夜,加辣根过氧化物酶标记的二抗(1:2 000稀释)37℃孵育1 h, DAB显色3-10 min,照片采用凝胶成像系统进行密度分析(Kodak system EDAS120)。

统计学处理 数据以均数±标准差表示,采用 F 检验和 q 检验进行统计学分析, $P<0.05$ 为差异有显著性。

2 结果

2.1 肝组织病理学及血清学改变 光镜下可见I/R组肝脏组织水肿、少量出血和大量炎性细胞浸润。PDTC预处理组肝组织充血、水肿减轻,仅见少量炎细胞浸润。肠缺血1 h再灌注2 h可引起血清ALT、AST水平明显升高,和对照组相比有显著差异($P<0.05$, $P<0.01$)。PDTC预处理组肝组织损伤明显改善,血清ALT、AST水平低于

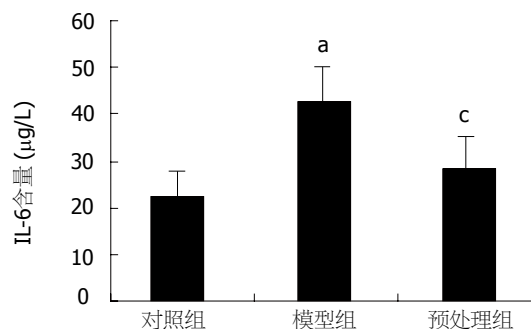


图2 PDTC对肠缺血再灌注大鼠血清IL-6含量的影响。模型组与对照组相比,^a $P<0.01$; PDTC预处理组与模型组相比,^b $P<0.05$ 。

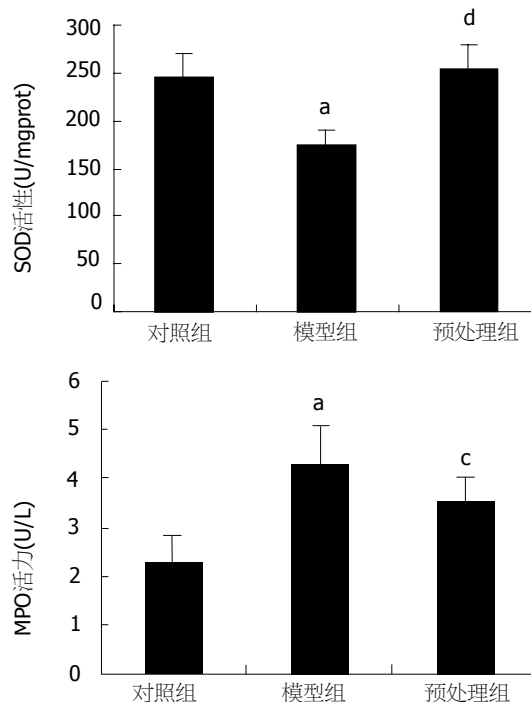


图3 PDTC对肠缺血再灌注大鼠肝组织SOD及MPO活性的影响。模型组与对照组相比,^a $P<0.01$; PDTC预处理组与模型组相比, $P<0.05$,^d $P<0.01$ 。

I/R组($P<0.01$, $P<0.05$, 图1)。I/R组的血清IL-6水平明显升高,与对照组相比差异显著($P<0.01$)。与I/R组比较,PDTC预处理组血清IL-6活性明显下降($P<0.05$, 图2)。

2.2 肝组织MPO、SOD及NF- κ B和P-selectin的表达 I/R组SOD及MPO水平分别出现明显下降和升高,与对照组相比有显著差异($P<0.01$, $P<0.01$),采用PDTC预处理后其水平分别较I/R组明显升高和下降($P<0.01$, $P<0.05$, 图3)。结果表明,PDTC可显著抑制肠缺血再灌注时远隔器官肝组织的白细胞浸润趋化和肝组织的ROS生成及脂质过氧化损伤。肝组织NF- κ B p65和P-selectin的表达在对照组中呈浅棕色,弱阳性表

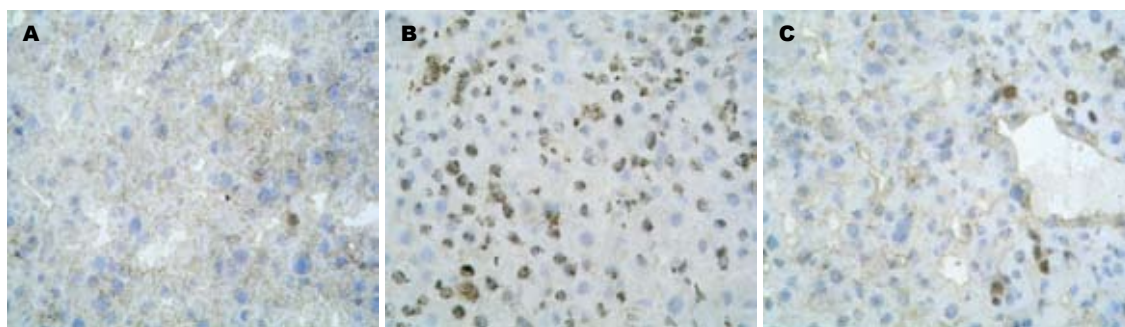


图 4 肠缺血再灌注大鼠肝脏NF- κ B p65蛋白表达. A: 对照组; B: 模型组; C: PDTC组.

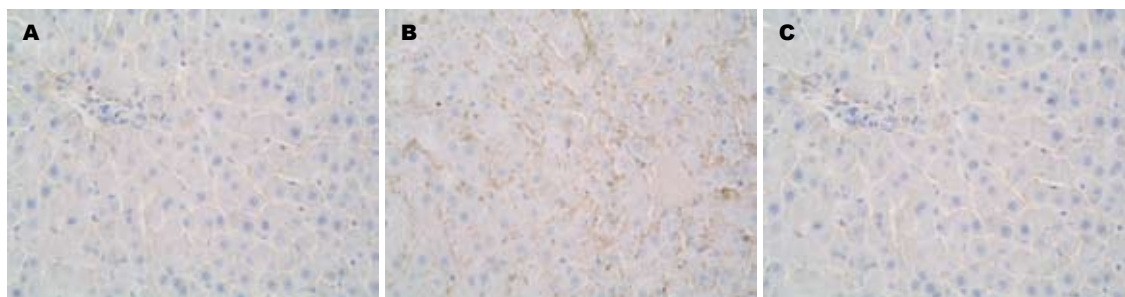


图 5 肠缺血再灌注大鼠肝脏P-selectin表达. A: 对照组; B: 模型组; C: PDTC组.

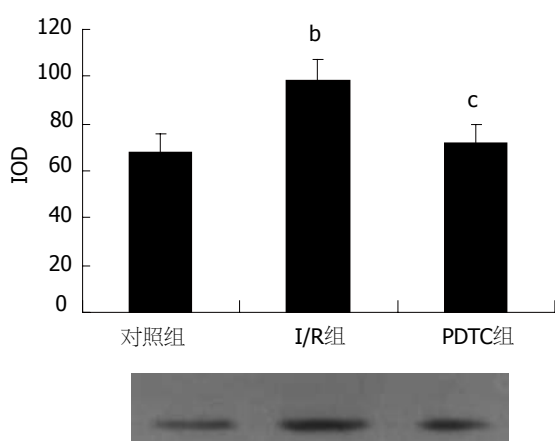


图 6 Western blot法测定大鼠肝脏NF- κ B p65蛋白表达密度值. 模型组与对照组相比, ^b $P < 0.01$; PDTC预处理组与模型组相比, ^c $P < 0.05$.

达, 主要集中于胞质中. 而模型组二者的表达不仅分布在胞质中, 在细胞核中也呈较强棕色表达. PDTC预处理组与模型组相比, NF- κ B p65和P-selectin的表达明显减弱(图4, 5). 对照组NF- κ B p65 Western blot分析的阳性信号较弱, 而在I/R组其表达明显增强($P < 0.01$). 与I/R组相比, PDTC预处理组NF- κ B p65表达明显减弱($P < 0.05$, 图6).

3 讨论

肠缺血再灌注损伤是严重创伤、休克等疾病治疗过程中经常发生的病理生理过程, 是多脏器功能衰竭发生、发展的重要原因. 肝脏是受

肠缺血再灌注损伤影响最直接的器官. IIR肝损伤的机制复杂, 早期研究认为许多损伤介质直接参与了IIR肝损伤的发病过程, 如ROS、NO、TNF- α 、细胞黏附因子及中性粒细胞浸润等直接对肝脏组织产生损伤^[5-7,13]. 近期研究表明, 这些介质的作用可受转录因子NF- κ B的调节^[8,14,15]. NF- κ B是与免疫球蛋白 κ 轻链基因增强子 κ B序列特异结合的核因子, 存在于细胞中. 由M_r50 000和M_r65 000两个亚单位组成, 二者通常与抑制蛋白I κ B结合, 这个磷酸化抑制体可被继发的泛素化(ubiquitin)而蛋白水解, NF- κ B被活化并转入细胞核, 进而转录激活目的基因. 激活的多形核白细胞和炎症因子被释放入血循环中, 与远隔脏器内的血管内皮细胞作用, 造成系统的炎症反应^[16-18]. 缺血再灌注后细胞黏附因子在白细胞所致的器官损伤中发挥重要的作用. 选择素是细胞黏附因子的一种, P-selectin的上调表达与损伤部位的白细胞黏附和浸润有关^[19-21]. 有研究表明, 严重的损伤可引起P-选择素转录水平的上调, 在P-selectin启动区受NF- κ B反应因子调控, NF- κ B活化可使P-selectin表达增加^[22]. 而MPO则主要存在于PMN中, 组织MPO活力可反映组织PMN的聚集量, 其活性可说明PMN在组织中的聚集情况^[23,24].

PDTC是一种抗氧化剂, 其可通过选择性抑制核因子- κ B的活化, 使致炎细胞因子基因表达受到抑制, 进而减少致炎细胞因子的释放^[25-27].

■名词解释

1 NF- κ B: 核转移因子 κ B, 是与免疫球蛋白 κ 轻链基因增强子 κ B序列特异结合的核因子, 存在于细胞中. 其被活化后转入细胞核, 进而转录激活目的基因.

2 PDTC: 脯氨酸二硫代氨基甲酸酯, 是一种抗氧化剂, 其可通过选择性抑制核因子- κ B的活化, 使致炎细胞因子基因表达受到抑制.

■同行评价

本文对大鼠肠缺血再灌注进行了研究,发现经PDTC预注射后可减弱此过程对肝脏的病理损伤,同时伴有P-selectin的表达增强及血清某些生化指标和细胞因子的变化。研究方法科学,结论正确,对肠缺血再灌注器官损伤的保护有指导意义。

我们发现,肠缺血1 h再灌注2 h可引起肝损伤,表现为血清ALT、AST水平升高并出现组织病理学改变如肝水肿、出血和白细胞浸润,肝组织SOD活性下降、MPO水平明显升高,血清IL-6含量明显高于对照组。这些改变与肝组织中NF- κ B p65和P-selectin的高表达相平行,这表明NF- κ B的活化上调中性粒细胞浸润和P-selectin的表达参与了IIR肝损伤的病理过程。PDTC预处理后,NF- κ B p65的表达水平明显下降,肝组织损伤减轻,血清ALT和AST、组织病理学、IL-6、PMN浸润等均明显改善,P-selectin的表达水平也明显下降。说明PDTC作为NF- κ B的抑制剂,通过抑制NF- κ B的活化,降低肝组织P-selectin和炎症细胞因子IL-6的表达,减轻了肝组织中性粒细胞的浸润,对肠缺血再灌注肝组织损伤具有明显的保护作用。

4 参考文献

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