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血必净对脓毒症早期大鼠急性肺损伤的作用及可能机制

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摘要 目的:探讨血必净注射液对脓毒症早期肺组织损伤的影响及其可能作用机制,为临床脓毒症时急性肺损伤的治疗提供新思路。**方法:**选用健康雄性 S-D 大鼠 30 只,随机分 3 组,即假手术组(Sham 组)、盐水组(NS 组)和血必净组(XBJ 组)。应用盲肠结扎穿孔法(CLP)建立脓毒症大鼠模型,术后 6 h 处死动物,通过 HE 染色光镜观察组织形态学改变及透射电镜观察肺组织超微结构,测定各组肺组织湿干重比,采用 RT-PCR 检测肺组织内皮素-1(ET-1)、诱导型一氧化氮合酶(iNOS)、基质金属蛋白酶-9(MMP-9)、组织金属蛋白酶抑制物-1(TIMP-1) mRNA 的表达。**结果:**XBJ 组肺泡及肺间质水肿和内皮细胞超微结构改变均较 NS 组明显减轻。术后 6 h,NS 组大鼠肺组织湿干重比(W/D)显著高于 Sham 组(5.37 ± 0.12 vs 4.33 ± 0.06 , $P < 0.01$),XBJ 组肺组织 W/D 较 NS 组显著降低(4.67 ± 0.09 vs 5.37 ± 0.12 , $P < 0.05$)。此外,XBJ 组大鼠肺组织 ET-1 (0.511 ± 0.111 vs 0.705 ± 0.122 , $P < 0.01$),iNOS (0.456 ± 0.075 vs 0.548 ± 0.098 , $P < 0.05$),MMP-9(0.617 ± 0.079 vs 0.732 ± 0.131 , $P < 0.05$),TIMP-1(0.438 ± 0.043 vs 0.515 ± 0.049 , $P < 0.01$) mRNA 的表达均较 NS 组明显降低,而 NS 组大鼠肺组织 ET-1 (0.705 ± 0.122 vs 0.400 ± 0.033 , $P < 0.01$),iNOS (0.548 ± 0.098 vs 0.334 ± 0.027 , $P < 0.01$),MMP-9 (0.732 ± 0.131 vs 0.352 ± 0.061 , $P < 0.01$),TIMP-1 mRNA (0.515 ± 0.049 vs 0.365 ± 0.068 , $P < 0.01$) 水平均较 Sham 组明显升高。**结论:**血必净治疗对脓毒症大鼠的肺组织损伤有一定的保护作用,可能与其降低肺组织 ET-1、iNOS、MMP-9、TIMP-1 的表达有关。

关键词:脓毒症;急性肺损伤;内皮素-1;诱导型一氧化氮合酶;基质金属蛋白酶-9;组织金属蛋白酶抑制物-1

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Effects and Mechanisms of Xuebijing on Early Sepsis Induced Acute Lung Injury in Rats

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ABSTRACT Objective: To investigate the effects and mechanisms of Xuebijing injection on early sepsis induced acute lung injury and explore a new therapy. **Methods:** Thirty healthy male Sprague-Dawley rats were randomly divided into 3 groups: Sham group(n=10), NS group (NS 4mL/kg n=10), Xuebijing Group(Xuebijing 4mL/kg n=10). Cecal ligation and puncture (CLP) was used to duplicate severe sepsis model. At 6h after CLP, the rats were sacrificed, the lungs were resected and histopathological characteristic was observed by transmission electron microscopy technique. The change of the lung wet/dry (W/D) weight ratio were studied. Meanwhile, the lungs were resected for detection of ET-1, iNOS, MMP-9, TIMP-1 mRNA with reverse transcription-polymerase chain reaction (RT-PCR). **Results:** The changes of pulmonary alveoli and the interstitial edema as well as lung tissue in Xuebijing group were better than those of NS group. The change of lung wet/dry (W/D) weight ratio in septic rats showed was significantly increased at 6 h after CLP (vs Sham group: 5.37 ± 0.12 vs 4.33 ± 0.06 , $P < 0.01$). The lung W/D was significantly decreased in XBJ group (vs NS group: 4.67 ± 0.09 vs 5.37 ± 0.12 , $P < 0.05$). The expressions in XBJ group lung tissue of ET-1(0.511 ± 0.111 vs 0.705 ± 0.122 , $P < 0.01$), iNOS(0.456 ± 0.075 vs 0.548 ± 0.098 , $P < 0.05$), MMP-9 (0.617 ± 0.079 vs 0.732 ± 0.131 , $P < 0.05$), TIMP-1 (0.438 ± 0.043 vs 0.515 ± 0.049 , $P < 0.01$) mRNA were significantly decreased than those in NS group. And the expressions of ET-1 (0.705 ± 0.122 vs 0.400 ± 0.033 , $P < 0.01$), iNOS (0.548 ± 0.098 vs 0.334 ± 0.027 , $P < 0.01$), MMP-9(0.732 ± 0.131 vs 0.352 ± 0.061 , $P < 0.01$), TIMP-1(0.515 ± 0.049 vs 0.365 ± 0.068 , $P < 0.01$) mRNA in NS group were significantly increased compared with those in the sham group. **Conclusions:** Xuebijing could protect against sepsis induced acute lung injury, which might be related with the decrease of ET-1, iNOS, MMP-9 and TIMP-1 mRNA expressions.

Key words: Sepsis; ALI; ET-1; iNOS; MMP-9; TIMP-1

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前言

脓毒症是危重患者的重要死亡原因之一,死亡率高达50-80%。脓毒症引起的ALI(acute lung injury)是导致脓毒症患者死亡率居高不下的主要原因之一,内皮功能障碍是其发生发展的重要环节。多种细胞因子及内皮衍生物对内皮功能的稳定性发挥着重要作用,对脓毒症的预后及转归有重要影响。多项国内外研究证实^[1]在脓毒症患者一些内皮衍生物及蛋白酶如内皮素-1(ET-1)/诱导型一氧化氮合酶(iNOS)、基质金属蛋白酶-9(MMP-9)/组织金属蛋白酶抑制物-1(TIMP-1)过度表达与失衡,使内皮系统受损,进一步提高了脓毒症的死亡率。血必净注射液具有降低内毒素水平、调节免疫及炎性介质、改善微循环、控制内皮损伤的作用^[2],对脓毒症的治疗有重要意义。本实验采用盲肠结扎穿孔(cecal ligation and puncture, CLP)法建立脓毒症大鼠模型,探讨血必净注射液对脓毒症时ALI的治疗疗效及肺组织内皮素-1(Endothelin-1, ET-1)、诱导型一氧化氮合酶(inducible nitric oxide synthase, iNOS)、基质金属蛋白酶-9(matrix metalloproteinase-9, MMP-9)、组织金属蛋白酶抑制物-1(tissue inhibitor of metalloproteinase, TIMP-1)mRNA水平的影响,以期为其临床应用提供更多的实验依据。

1 材料与方法

1.1 制模及分组

应用盲肠结扎穿孔法(CLP)建立脓毒症模型,由河北医科大学实验动物中心提供健康清洁雄性体重250~300 g S-D大鼠30只,随机分3组,假手术组(Sham)、盐水组(NS)及血必净组(XBJ),每组10只。1周适应性饲养,10%水合氯醛(350 mg/kg)腹腔注射麻醉,常规消毒铺巾,颈动脉置管,腹正中线开腹暴露盲肠,距盲端1 cm处结扎盲肠,针头贯穿肠管并留置一橡皮条贯通防止肠管闭合,挤出少量肠内容物,逐层缝合关腹,术毕腹部皮下注射生理盐水30ml/kg补充术中液体丢失量,编号入

笼。XBJ组CLP术后给予血必净注射液4 mL/kg尾静脉注射,Sham组只开腹,不结扎和穿孔盲肠。制模完成后6h由颈总动脉采血处死动物。

1.2 观察内容及检测方法

CLP术后6h处死动物,剪取右肺下叶1×1×3立方毫米大小以4%戊二醛固定,电镜检查。10%多聚甲醛固定标本,梯度乙醇脱水,石蜡包埋切片,HE染色后光镜观察。留取各组大鼠右肺中叶称湿重,吸干水分后,置于85℃烤箱中24小时至恒重,称干重,计算肺湿干重比(W/D)。RT-PCR半定量分析ET-1mRNA、iNOSmRNA以及MMP-9mRNA、TIMP-1mRNA水平,进行组织总RNA的提取,逆转录,扩增产物检测,严格按照试剂盒说明书步骤进行。

1.3 统计学分析

采用SPSS19.0版软件包(SPSS Company, Chicago, Illinois, USA)进行统计学分析,多组间计量资料比较采用单因素方差分析,两组间比较采用t检验,以P<0.05为差异有统计学意义。

2 结果

2.1 CLP术后一般情况的观察

Sham组大鼠麻醉清醒后活动基本如常,剖腹未见脓臭渗出,组织脏器无明显充血水肿;NS组和XBJ组大鼠术后观察发现大鼠麻醉清醒的时限延长,有呼吸频促、意识委顿、竖毛寒颤、饮食低迷等表现,剖腹可见肠管充血肿胀,脓血性腹腔积液、浑浊恶臭等脓毒症腹腔感染症状,NS组大鼠上述脓毒症表现尤重。

2.2 肺组织HE染色及光镜下观察

光镜下,Sham组肺泡结构清晰完整,肺泡间隔无明显异常;XBJ组肺泡结构虽尚完整,但肺泡腔可见中性粒细胞浸润,肺间质充血水肿,肺泡间隔增宽;NS组肺组织改变程度更重,肺泡结构破坏,肺泡腔可见中性粒细胞浸润明显,部分肺不张,甚至肺气肿等代偿性病变。(图1)。

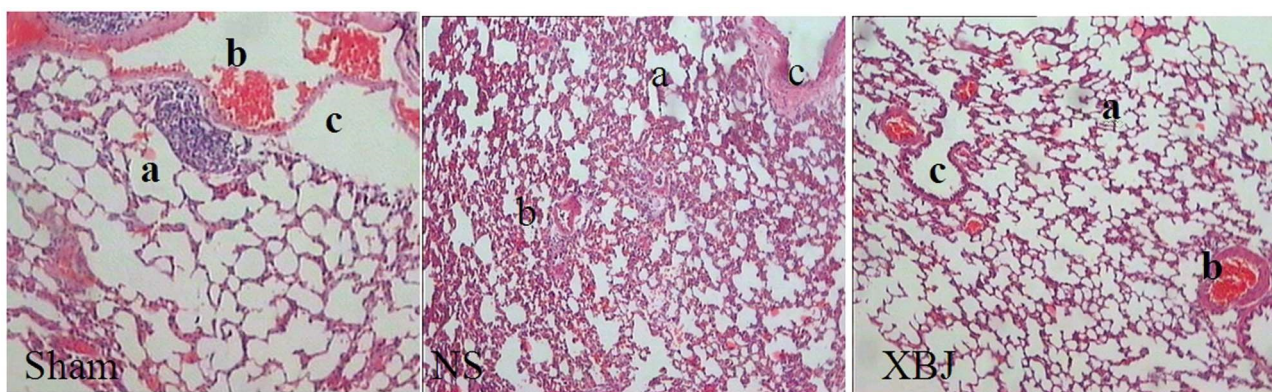


Fig.1 The pathological change of lung tissue in Sham, NS, XBJ group (HE stain×200). Abbreviations: a, alveoli; b, blood vessels; c, terminal bronchioles

2.3 电镜观察

Sham组电镜下肺组织内皮细胞边界清,细胞连接紧密,肺泡上皮细胞及毛细血管内皮细胞基底膜完整,胞内罕见吞饮小泡;XBJ组细胞连接欠规整,可见内皮细胞间隙,内皮细胞胞浆内有少量吞饮小泡,肺泡腔可见炎细胞浸润,线粒体轻度肿胀,

而NS组组织改变明显重于XBJ组,电镜可见其细胞连接模糊不清,基底膜破坏,胞浆内可见数量众多的吞饮小泡。(图2)。

2.4 三组大鼠术后6h肺组织湿干重比(W/D)比较

术后6小时,NS组大鼠肺组织W/D比与Sham组相比显著增加(5.37 ± 0.12 vs 4.33 ± 0.06 , $P < 0.01$),XBJ组肺组织W/D

比 NS 组显著减少 (4.67 ± 0.09 vs 5.37 ± 0.12 , $P < 0.05$), 且 XBJ 组肺组织 W/D 比 Sham 组增加 (4.67 ± 0.09 vs 4.33 ± 0.06 , $P < 0.05$)。

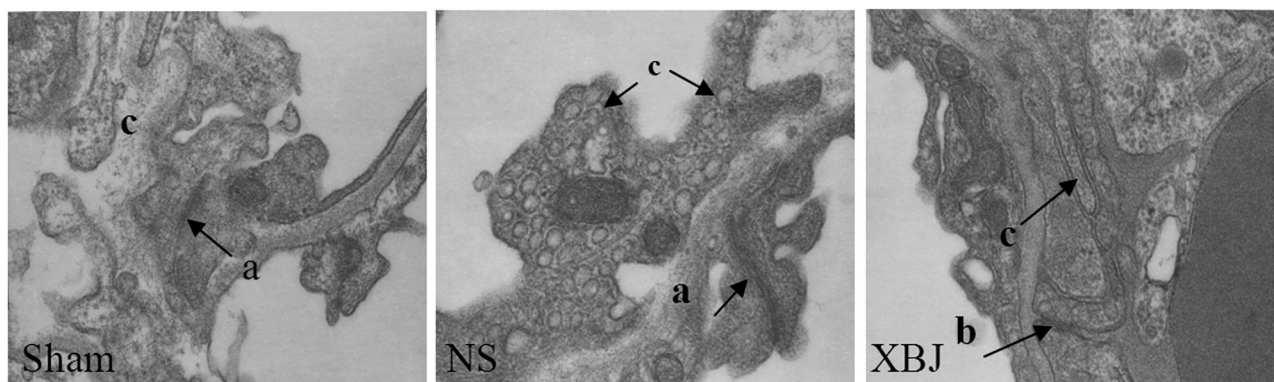


Fig.2 Pathological change of lung vascular endothelial cell in Sham, NS, XBJ group under transmission electron microscopy (× 3000).

Abbreviations: a, intercellular junction; b, endothelial gap; c, swallowing vesicle

2.5 三组大鼠肺组织 ET-1、iNOS、MMP-9、TIMP-1 mRNA 水平比较
NA 水平明显高于 Sham 组 ($P < 0.05$ 或 $P < 0.01$), 而 XBJ 组以上指标均显著低于 NS 组 ($P < 0.05$ 或 $P < 0.01$)。

NS 组及 XBJ 组肺组织 ET-1、iNOS、MMP-9、TIMP-1 mRNA

表 1 三组大鼠肺组织 ET-1、iNOS、MMP-9、TIMP-1 mRNA 表达比较 ($n=10$, $\bar{x} \pm s$, mRNA/ β -action)

Table 1 Comparison of the ET-1, iNOS, MMP-9 and TIMP-1 mRNA expressions in the lung tissue between three groups

Group	ET-1 mRNA	iNOS mRNA	MMP-9 mRNA	TIMP-1 mRNA
Sham group	0.400 ± 0.033	0.334 ± 0.027	0.352 ± 0.061	0.365 ± 0.068
NS group	0.705 ± 0.122^d	0.548 ± 0.098^d	0.732 ± 0.131^d	0.515 ± 0.049^d
XBJ group	0.511 ± 0.111^{ac}	0.456 ± 0.075^{bd}	0.617 ± 0.079^{bd}	0.438 ± 0.043^{ad}

Note: compared with NS group ^a $P < 0.01$, ^b $P < 0.05$; compared with Sham group, ^c $P < 0.05$, ^d $P < 0.01$.

3 讨论

脓毒症通常指主要由多种细菌感染引起的系统炎症反应综合征^[3],死亡率高达 50-80%。内皮系统功能障碍是脓毒症发展恶化的中心环节,近期研究表明内皮功能障碍可能是早期预测脓毒症 MODS 发生率和死亡率的独立危险因素^[4]。内皮祖细胞移植可以显著降低肺、肝、肾组织损伤和干湿重比^[5]。近年来,内皮系统在重症脓毒症中所扮演的角色与病程进展中的作用是研究热点^[6]。

内皮系统的稳定性需多种介质的共同作用,内皮素-1(Endothelin-1, ET-1)是强有效的内源性血管收缩剂,主要由内皮细胞分泌,其通过 ETA 和 ETB 两种类型受体而发挥作用。最近的研究表明 ET-1 在转录因子如 NF- κ B 的活化和一些细胞因子 TNF- α ^[7]、IL-1、IL-6 的表达中也起着促进作用^[8],可以导致血管细胞纤维化和刺激产生活性氧,从而启动促炎机制^[9],其在脓毒症所致急性肺损伤(SA-ALI)中的作用已被证实^[10]。血浆 ET-1 水平在不同的脓毒症动物实验中都是增加的,更有一些研究显示 ET-1 与心肌损伤及脓毒性休克患者死亡率具有相关性^[11],ET-1 在脓毒症以及脓毒症所致脏器损伤中的作用已得到越来越多的研究证实^[12]。脓毒症炎症反应无法控制时,大量炎症因子释放入血,内毒素诱导多种细胞内诱导型一氧化氮合酶(iNOS)表达^[13],活化的 iNOS 可产生高浓度的 NO,而高浓度的 NO 成为一把双刃剑,决定了疾病的进展方向。高浓度 NO 起直接

杀菌作用,也可以舒张血管,降低血压,导致血浆外渗通过激活血管内皮细胞,成为激发全身炎症反应的主要炎症介质,最终加剧低血压导致休克,选择性 iNOS 的抑制剂可以降低脓毒症大鼠死亡率^[14]。MMP-9 与 TIMP-1 同样也在脓毒症的发生发展过程中发挥非常重要的作用^[15]。基质金属蛋白酶(MMPs)是一类由锌离子作为辅酶的内切蛋白水解酶家族,一些研究表明脓毒症时 MMP 水平明显升高^[16],被激活后主要降解明胶和基底膜的主要成分 IV 型胶原,从而破坏血管基底膜及组织结构,促进内皮细胞凋亡,产生不可逆的损伤。组织金属蛋白酶抑制物(TIMPs)是 MMPs 的天然抑制剂,其中 TIMP-1 是 MMP-9 的天然抑制剂,同时近年来,医学界对 TIMP-1 在重症脓毒症预后的预测性意义越来越得到重视。一些临床研究表明在重症脓毒症患者中,存活患者的 TIMP-1 浓度明显高于死亡患者^[17],提示 TIMP-1 与重症脓毒症患者的存活率具有相关性^[18,19]。有研究表明因脓毒性休克而死亡的患者治疗中 TIMP-1/MMP-9 比值持续高于获救者,且与死亡率密切相关^[20],并可作为新的生物标记物判断脓毒症预后。

本研究结果显示经血必净注射液干预的脓毒症大鼠的肺组织损伤明显改善,ET-1、iNOS、MMP-9 及 TIMP-1 的表达水平明显下降,提示通过血必净治疗对脓毒症大鼠的肺组织损伤有一定的保护作用,可能与其降低肺组织 ET-1、iNOS、MMP-9、TIMP-1 的表达,减轻血管内皮细胞损伤,维持内皮系统平衡和维持基质稳定性有关。当然,本研究还存在一些不足,如能增加

多次观察时间点, 可比较不同时间窗脓毒症组织脏器的变化, 进一步完善研究的全面性, 为今后脓毒症的临床治疗及方案的选择提供更有力的佐证。

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