

TREM-1 抑制肽 LR12 对脓毒症小鼠心肌损伤的改善作用 及其机制

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[摘 要] **目的:** 探讨髓系细胞触发受体 1 (TREM-1) 抑制肽 LR12 对脓毒症小鼠心肌损伤的作用, 并阐明其可能的作用机制。**方法:** 采用盲肠结扎穿刺术 (CLP) 建立脓毒症小鼠模型。将 40 只雄性小鼠随机分为假手术组、模型组、CLP+LR12 对照肽 (LR12-scr) 组、CLP+LR12 组和 CLP+DNase I 组, 每组 8 只。除假手术组外, 其余各组小鼠均行 CLP 建立脓毒症模型, 术后 1 h 分别腹腔注射 LR12-scr、LR12 和 DNase I。术后 24 h, 观察各组小鼠状态和行为表现。采用小动物超声系统检测各组小鼠心脏功能, 酶联免疫吸附试验 (ELISA) 法检测各组小鼠血清中血清肌酸激酶同工酶 (CK-MB) 和心肌肌钙蛋白 I (cTnI) 及炎症指标白细胞介素 1 β (IL-1 β)、白细胞介素 6 (IL-6) 和肿瘤坏死因子 α (TNF- α) 水平以及心肌组织中髓过氧化物酶 (MPO)-DNA (MPO-DNA) 和中性粒细胞弹性蛋白酶 (NE)-DNA (NE-DNA) 水平, HE 染色法观察各组小鼠心肌组织病理形态表现, 免疫荧光染色法检测各组小鼠心肌组织中淋巴细胞抗原 6G (Ly6G) 和瓜氨酸化组蛋白 H3 (cit-H3) 蛋白共表达情况, Western blotting 法检测各组小鼠心肌组织中 TREM1、MPO、NE 和 cit-H3 蛋白表达水平。**结果:** 与假手术组比较, 模型组小鼠精神萎靡, 心肌组织排列紊乱, 心肌细胞萎缩、变形; 与模型组比较, CLP+LR12 组和 CLP+DNase I 组小鼠精神状态及心肌组织损伤明显改善, CLP+LR12-scr 组无明显变化。心脏功能检测, 与假手术组比较, 模型组小鼠左心室射血分数 (LVEF) 和左心室短轴缩短率 (LVFS) 明显降低 ($P<0.01$); 与模型组比较, CLP+LR12 组和 CLP+DNase I 组小鼠 LVEF 及 LVFS 明显升高 ($P<0.01$)。ELISA 法, 与假手术组比较, 模型组小鼠血清中 CK-MB 和 cTnI 水平及 IL-1 β 、IL-6 和 TNF- α 水平均明显升高 ($P<0.01$), 心肌组织中 MPO-DNA 和 NE-DNA 水平明显升高 ($P<0.01$); 与模型组比较, CLP+LR12 组和 CLP+DNase I 组小鼠血清中 CK-MB 及 cTnI 水平以及 IL-1 β 、IL-6 和 TNF- α 水平明显降低 ($P<0.01$), 心肌组织中 MPO-DNA 和 NE-DNA 水平明显降低 ($P<0.01$); CLP+LR12-scr 组上述指标均无明显变化 ($P>0.05$)。与假手术组比较, 模型组小鼠心肌组织中 Ly6G 和 cit-H3 蛋白共表达增加; 与模型组比较, CLP+LR12 组和 CLP+DNase I 组小鼠心肌组织中 Ly6G 及 cit-H3 蛋白共表达减少。Western blotting 法, 与假手术组比较, 模型组小鼠心肌组织中 TREM-1、MPO、NE 和 cit-H3 蛋白表达水平均明显升高 ($P<0.01$); 与模型组比较, CLP+LR12 组小鼠心肌组织中 TREM-1、MPO、NE 和 cit-H3 蛋白表达水平明显降低 ($P<0.01$), CLP+DNase I 组小鼠心肌组织中 MPO、NE 和 cit-H3 蛋白表达水平明显降低 ($P<0.01$), TREM-1 蛋白表达水平差异无统计学意义 ($P>0.05$)。**结论:** TREM-1 抑制肽 LR12 可改善脓毒症小鼠的心功能, 减轻心肌炎症和损伤, 其机制可能与抑制中性粒细胞胞外诱捕网 (NETs) 形成有关。

[关键词] 脓毒症; 心肌损伤; 髓系细胞触发受体 1; 中性粒细胞胞外诱捕网; LR12

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Improvement effect of TREM-1 inhibitory peptide LR12 on myocardial injury in septic mice and its mechanism

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ABSTRACT Objective: To discuss the effect of triggering receptor expressed on myeloid cells-1 (TREM-1) inhibitory peptide LR12 on myocardial injury in the septic mice, and to clarify its possible mechanism. **Methods:** The mouse model of sepsis was established by cecal ligation and puncture (CLP). Forty male mice were randomly divided into sham operation group, model group, CLP+LR12 control peptide (LR12-scr) group, CLP+LR12 group, and CLP+DNase I group, and there were 8 mice in each group. Except for sham operation group, the mice in the other groups underwent CLP to establish the sepsis models, and were intraperitoneally injected with LR12-scr, LR12, and DNase I at 1 h after operation, respectively. At 24 h after operation, the status and behavior of the mice in various groups were observed. A small animal ultrasound system was used to detect the cardiac function of the mice in various groups; enzyme-linked immunosorbent assay (ELISA) method was used to detect the levels of creatine kinase isoenzyme-MB (CK-MB), cardiac troponin I (cTnI), and inflammatory indicators interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) in serum, as well as the levels of myeloperoxidase (MPO)-DNA and neutrophil elastase (NE)-DNA in myocardium tissue of the mice in various groups; HE staining was used to observe the pathomorphology of myocardium tissue of the mice in various groups; immunofluorescence staining was used to detect the co-expression of lymphocyte antigen 6G (Ly6G) and citrullinated histone H3 (cit-H3) proteins in myocardium tissue of the mice in various groups; Western blotting method was used to detect the expression levels of TREM-1, MPO, NE, and cit-H3 proteins in myocardium tissue of the mice in various groups. **Results:** Compared with sham operation group, the mice in model group were listless, with disordered arrangement of myocardium tissue, and atrophy and deformation of cardiomyocytes; compared with model group, the mental state and myocardium tissue injury of the mice in CLP+LR12 group and CLP+DNase I group were significantly improved, while no significant change was observed in CLP+LR12-scr group. The cardiac function detection results showed that compared with sham operation group, the left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) of the mice in model group were significantly decreased ($P < 0.01$); compared with model group, the LVEF and LVFS of the mice in CLP+LR12 group and CLP+DNase I group were significantly increased ($P < 0.01$). The ELISA results showed that compared with sham operation group, the levels of CK-MB, cTnI, IL-1 β , IL-6, and TNF- α in serum and the levels of MPO-DNA and NE-DNA in myocardium tissue of the mice in model group were significantly increased ($P < 0.01$); compared with model group, the levels of CK-MB, cTnI, IL-1 β , IL-6, and TNF- α in serum and the levels of MPO-DNA and NE-DNA in myocardium tissue of the mice in CLP+LR12 group and CLP+DNase I group were significantly decreased ($P < 0.01$); no significant changes in the above indicators were observed in CLP+LR12-scr group ($P > 0.05$). The immunofluorescence staining results showed that compared with sham operation group, the co-expression of Ly6G and cit-H3 proteins in myocardium tissue of the mice in model group was increased; compared with model group, the co-expression of Ly6G and cit-H3

proteins in myocardium tissue of the mice in CLP+LR12 group and CLP+DNase I group was decreased. The Western blotting method results showed that compared with sham operation group, the expression levels of TREM-1, MPO, NE, and cit-H3 proteins in myocardium tissue of the mice in model group were significantly increased ($P<0.01$); compared with model group, the expression levels of TREM-1, MPO, NE, and cit-H3 proteins in myocardium tissue of the mice in CLP+LR12 group were significantly decreased ($P<0.01$), while the expression levels of MPO, NE, and cit-H3 proteins in myocardium tissue of the mice in CLP+DNase I group were significantly decreased ($P<0.01$), and there was no significant difference in the expression level of TREM-1 protein ($P>0.05$). **Conclusion:** TREM-1 inhibitory peptide LR12 can improve the cardiac function and alleviate myocardial inflammation and injury in the septic mice, and its mechanism may be related to inhibiting the formation of neutrophil extracellular traps (NETs).

KEYWORDS Sepsis; Myocardial injury; Triggering receptor expressed on myeloid cells-1; Neutrophil extracellular trap; LR12

脓毒症是由感染引发的全身性炎症反应失调导致的致命性器官功能障碍疾病, 心肌损伤是其常见并发症, 明显增加患者死亡风险。中性粒细胞作为固有免疫系统的核心效应细胞, 是抗感染的第一道防线, 通过吞噬、脱颗粒作用和形成中性粒细胞胞外诱捕网(neutrophil extracellular traps, NETs)等机制, 在限制细菌及病毒感染扩散中发挥关键作用^[1-2]。NETs是由DNA、颗粒蛋白[如髓过氧化物酶(myeloperoxidase, MPO)和中性粒细胞弹性蛋白酶(neutrophil elastase, NE)]及组蛋白等构成的网状结构, 是多种病理条件下免疫应答的关键成分。然而, NETs过度产生可能诱发脓毒症中的组织损伤, 通过增加炎症因子分泌和促进多器官细胞死亡而加速疾病进展^[3-4]。髓系细胞触发受体1(triggering receptor expressed on myeloid cells-1, TREM-1)是免疫球蛋白超家族的重要成员, 主要表达于中性粒细胞和单核细胞及巨噬细胞等髓系细胞表面, 在炎症信号放大和免疫识别协同中发挥关键调控功能。研究^[5-6]发现: TREM-1的异常活化不仅与脓毒症和肺结核等疾病的发病机制有密切关联, 还可通过促进NETs释放及其下游信号通路的激活, 参与器官损伤过程。LR12是一种特异性靶向TREM-1受体的小分子多肽抑制剂, 可通过诱导可溶性TREM-1(soluble TREM-1, sTREM-1)释放并竞争性结合其配体, 有效抑制TREM-1信号传导, 从而抑制过度炎症反应^[7]。目前, TREM-1抑制肽LR12已在感染性休克患者的Ⅱa期临床试验中显示出良好的安全性和耐受性, 但其对脓毒症后心肌损伤的作用尚未明确。本研究拟通过建立脓毒症小鼠模型, 探讨TREM-1抑制肽LR12对脓毒症小鼠心肌损伤的作用及其可能的机

制, 以期为脓毒症性心肌损伤的防治提供新思路。

1 材料与方法

1.1 实验动物、主要试剂和仪器 SPF级雄性C57BL/6J小鼠40只, 8周龄, 体质量20~22 g, 购自河北省实验动物中心, 动物生产许可证号: SCXK(冀)2021-002。小鼠饲养于恒温、恒湿且明暗循环的环境, 可自由进食饮水。LR12和LR12对照肽LR12-scramble(LR12-scr)购自南京正肽生化科技有限公司, NETs抑制剂DNase I购自北京索莱宝科技有限公司, 肌酸激酶同工酶-MB(creatine kinase isoenzyme-MB, CK-MB)、心肌肌钙蛋白I(cardiac troponin I, cTnI)、白细胞介素 1β (interleukin- 1β , IL- 1β)、白细胞介素6(interleukin-6, IL-6)、肿瘤坏死因子 α (tumor necrosis factor- α , TNF- α)、MPO-DNA和NE-DNA酶联免疫吸附试验(enzyme-linked immunosorbent assay, ELISA)检测试剂盒均购自上海酶联生物科技有限公司, TREM-1、MPO、NE和淋巴细胞抗原6G(lymphocyte antigen 6G, Ly6G)抗体均购自武汉三鹰生物技术有限公司, 瓜氨酸化组蛋白H3(citrullinated histone H3, cit-H3)抗体购自美国Cell Signaling Technology公司。VINNOD8 LAB小动物超声成像系统购自北京益仁恒业科技有限公司, Mini-PROTEAN 3电泳系统购自美国Bio-Rad公司, IX51倒置白光/荧光拍照显微镜购自日本奥林巴斯公司。

1.2 实验动物分组、脓毒症小鼠模型制备和处理

适应性饲养1周后, 将40只小鼠随机分为假手术组、模型组、盲肠结扎穿刺术(cecum ligation and puncture, CLP)+LR12-scr组、CLP+LR12组

和CLP+DNase I组,每组8只。除假手术组外,其余各组小鼠均采用CLP建立脓毒症小鼠模型^[8],具体操作如下:术前禁食12 h后,腹腔注射戊巴比妥钠($50\text{ mg}\cdot\text{kg}^{-1}$)以麻醉小鼠,并以仰卧位固定于无菌操作台。于腹中线左侧0.5 cm处作1 cm纵行切口,打开腹腔,使盲肠暴露,在其长度1/2处进行结扎。使用22号针头在盲肠远端进行2次贯穿性穿刺,注意避开血管,轻压挤出少量肠内容物后回纳盲肠,逐层缝合腹壁切口。术后于颈部皮下注射1 mL 37℃生理盐水补充体液。假手术组小鼠仅行开腹、关腹操作,不进行结扎和穿刺。术后1 h,CLP+LR12-scr组小鼠腹腔注射 $5\text{ mg}\cdot\text{kg}^{-1}$ LR12-scr,CLP+LR12组小鼠腹腔注射 $5\text{ mg}\cdot\text{kg}^{-1}$ LR12^[9],CLP+DNase I组小鼠腹腔注射 $10\text{ mg}\cdot\text{kg}^{-1}$ DNase I^[10],假手术组和模型组小鼠腹腔注射等体积生理盐水。观察并记录各组小鼠术后行为表现和存活情况。

1.3 小动物超声成像系统检查各组小鼠心脏功能 于CLP术后24 h麻醉各组小鼠,以仰卧位固定于操作台,剃除前胸毛发。采用小动物超声成像系统,于左心室长轴切面采集各组小鼠M型超声心动图像,测定小鼠左心室射血分数(left ventricular ejection fraction, LVEF)和左心室短轴缩短率(left ventricular fraction shortening, LVFS)。

1.4 试剂盒检测各组小鼠血清中CK-MB、cTnI、IL-1 β 、IL-6和TNF- α 水平 心脏超声检测结束后,各组小鼠进行眼眶取血,血液样本离心后收集上清。严格按照ELISA试剂盒说明书操作,检测各组小鼠血清中心肌损伤标志物CK-MB和cTnI水平及炎症因子IL-1 β 、IL-6和TNF- α 水平。

1.5 试剂盒检测各组小鼠心肌组织中MPO-DNA和NE-DNA水平 取血结束后,采用颈椎脱臼法处死各组小鼠,迅速摘取心脏。使用磷酸盐缓冲液(phosphate buffered saline, PBS)冲洗残留血液后,取部分心肌组织进行匀浆。组织匀浆液离心后,收集上清液。严格按照试剂盒说明书操作,检测各组小鼠心肌组织中MPO-DNA和NE-DNA水平。

1.6 HE染色法观察各组小鼠心肌组织病理形态表现 取各组小鼠心肌组织,置于4%多聚甲醛固定24 h,常规脱水、石蜡包埋,制成厚度为4 μm 的心肌组织石蜡切片。HE染色后封片,于显微镜下观察心肌组织病理形态表现并采集图像。

1.7 免疫荧光染色法检测各组小鼠心肌组织中Ly6G和cit-H3蛋白共表达情况 将各组小鼠心肌组织,置于4%多聚甲醛中固定24 h,最佳切割温度(optimal cutting temperature, OCT)化合物包埋后制备切片。切片经0.5% Triton X-100透化处理15 min,10%山羊血清室温封闭2 h,加入Ly6G和cit-H3一抗(1:500稀释),4℃孵育过夜。PBS缓冲液漂洗3次后,避光条件下加入荧光标记二抗,室温孵育2 h。弃去二抗后,滴加DAPI染核15 min,PBS缓冲液清洗3次后封片。于荧光显微镜下观察心肌组织中Ly6G和cit-H3蛋白共表达情况并采集图像。Ly6G用于定位中性粒细胞在组织中的浸润与分布,cit-H3是NETs形成的关键核心标志物。Ly6G和cit-H3蛋白阳性染色代表NETs形成,二者共表达越强,提示心肌组织中中性粒细胞中NETs形成越多。

1.8 Western blotting法检测各组小鼠心肌组织中TREM-1、MPO、NE和cit-H3蛋白表达水平 取各组小鼠心肌组织,加入放射免疫沉淀法(radio immunoprecipitation assay, RIPA)裂解液匀浆,离心取上清,二喹啉甲酸(bicinchoninic acid, BCA)试剂盒检测蛋白浓度。取等量蛋白进行十二烷基硫酸钠-聚丙烯酰胺凝胶电泳(sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE)后转膜,5%脱脂奶粉封闭。分别加入TREM-1、MPO、NE、cit-H3和 β -actin一抗工作液(1:1 000稀释)后置于4℃冰箱中孵育过夜。加入辣根过氧化物酶(horseradish peroxidase, HRP)标记的山羊抗兔二抗(1:500稀释),室温孵育1 h。增强型化学发光法(enhanced chemiluminescence, ECL)试剂显影成像,采用Image J软件分析蛋白条带灰度值,以 β -actin为内参,计算目的蛋白表达水平。目的蛋白表达水平=目的蛋白条带灰度值/内参蛋白条带灰度值。

1.9 统计学分析 采用SPSS 26.0软件进行统计学分析。各组小鼠LVEF和LVFS,血清中CK-MB、cTnI、IL-1 β 、IL-6和TNF- α 水平,心肌组织中MPO-DNA和NE-DNA水平以及TREM-1、MPO、NE和cit-H3蛋白表达水平均符合正态分布,以 $\bar{x}\pm s$ 表示,多组间样本均数比较采用单因素方差分析,组间样本均数两两比较采用LSD-*t*检验。以 $P<0.05$ 为差异有统计学意义。

2 结 果

2.1 各组小鼠术后行为表现和存活情况 假手术组小鼠呼吸频率正常，毛发整洁光滑，眼眶无明显分泌物，对外界刺激（如触碰和声音等）反应迅速。模型组和 CLP+LR12-scr 组小鼠呼吸急促，精神萎靡，行动迟缓，眼眶分泌物增加，毛发杂乱，背部竖毛较多，对外界刺激反应迟钝。CLP+LR12 组和 CLP+DNase I 组小鼠呼吸频率接近正常，眼眶分泌物和背部竖毛减少，对外界刺激反应较快。术后 24 h，假手术组无小鼠死亡，模型组小鼠死亡 3 只，CLP+LR12 组和 CLP+DNase I 组小鼠各死亡 1 只，CLP+LR12-scr 组小鼠死亡 2 只。各组小鼠出现死亡则及时给予补充，保证每组 8 只。

2.2 各组小鼠心脏功能 与假手术组比较，模型组小鼠 LVEF 和 LVFS 均明显降低 ($P<0.01$)；与模型组比较，CLP+LR12 组和 CLP+DNase I 组小鼠 LVEF 及 LVFS 均明显升高 ($P<0.01$)，CLP+LR12-scr 组小鼠 LVEF 和 LVFS 差异无统计学意义

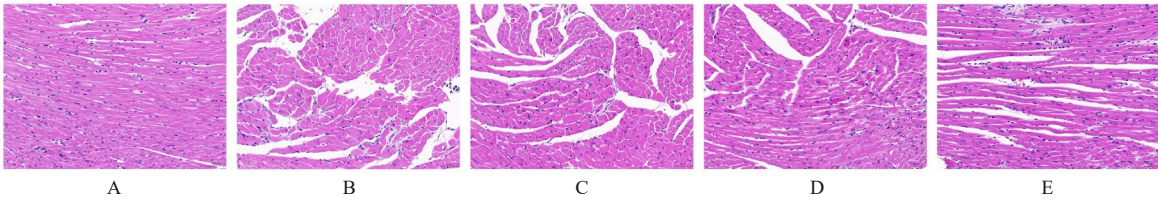
($P>0.05$)。见表 1。

表 1 各组小鼠心脏功能
Tab. 1 Cardiac function of mice in various groups
($n=8, \bar{x}\pm s, \eta/\%$)

Group	LVEF	LVFS
Sham operation	66.85±3.17	37.28±3.06
Model	45.19±2.26*	26.34±2.40*
CLP+ LR12-scr	46.72±2.05	27.10±1.82
CLP+LR12	57.26±2.83 [△]	31.13±2.47 [△]
CLP+DNase I	61.09±3.29 [△]	34.84±2.11 [△]

* $P<0.01$ compared with sham operation group; [△] $P<0.01$ compared with model group.

2.3 各组小鼠心肌组织病理形态表现 假手术组小鼠心肌组织排列规整，心肌细胞形态正常；模型组和 CLP+LR12-scr 组小鼠心肌组织排列紊乱，心肌细胞萎缩、变形，可见炎性细胞浸润；CLP+LR12 组和 CLP+DNase I 组小鼠心肌组织排列较规整，细胞形态明显改善，炎性细胞浸润减少。见图 1。



A: Sham operation group, B: Model group, C: CLP+LR12-scr group, D: CLP+LR12 group, E: CLP+DNase I group.

图 1 各组小鼠心肌组织病理形态表现(HE, ×200)

Fig. 1 Pathomorphology of myocardium tissue of mice in various groups (HE, ×200)

2.4 各组小鼠血清中 CK-MB、cTnI、IL-1 β 、IL-6 和 TNF- α 水平 与假手术组比较，模型组小鼠血清中 CK-MB 和 cTnI 水平均明显升高 ($P<0.01$)；与模型组比较，CLP+LR12 组和 CLP+DNase I 组小鼠血清中 CK-MB 及 cTnI 水平均明显降低 ($P<0.01$)，CLP+LR12-scr 组小鼠血清中 CK-MB 和 cTnI 水平差异无统计学意义 ($P>0.05$)。见表 2。

与假手术组比较，模型组小鼠血清中 IL-1 β 、IL-6 和 TNF- α 水平均明显升高 ($P<0.01$)；与模型组比较，CLP+LR12 组和 CLP+DNase I 组小鼠血清中 IL-1 β 、IL-6 及 TNF- α 水平均明显降低 ($P<0.01$)；CLP+LR12-scr 组小鼠血清中 IL-1 β 、IL-6 和 TNF- α 水平差异无统计学意义 ($P>0.05$)。见表 3。

2.5 各组小鼠心肌组织中 MPO-DNA 和 NE-DNA 水平 与假手术组比较，模型组小鼠心肌组织中

表 2 各组小鼠血清中 CK-MB 和 cTnI 水平

Tab. 2 Serum levels of CK-MB and cTnI of mice in various groups
($n=8, \bar{x}\pm s$)

Group	CK-MB [$\lambda_B/(U\cdot L^{-1})$]	cTnI [$\rho_B/(ng\cdot L^{-1})$]
Sham operation	28.73±1.86	19.21±1.52
Model	57.65±4.18*	53.37±4.83*
CLP+LR12-scr	58.43±4.35	51.82±4.70
CLP+LR12	40.08±3.07 [△]	35.88±2.14 [△]
CLP+DNase I	34.23±2.79 [△]	29.43±2.53 [△]

* $P<0.01$ compared with sham operation group; [△] $P<0.01$ compared with model group.

MPO-DNA 和 NE-DNA 水平均明显升高 ($P<0.01$)；与模型组比较，CLP+LR12 组和 CLP+DNase I 组小鼠心肌组织中 MPO-DNA 及 NE-DNA 水平明显

表3 各组小鼠血清中IL-1β、IL-6和TNF-α水平
Tab.3 Serum levels of IL-1β, IL-6 and TNF-α of mice in various groups [n=8, $\bar{x}\pm s$, $\rho_B/(\text{ng}\cdot\text{L}^{-1})$]

Group	IL-1β	IL-6	TNF-α
Sham operation	34.08±3.81	12.34±1.05	110.20±9.72
Model	83.05±6.24*	33.18±2.16*	188.62±12.65*
CLP+LR12-scr	82.46±7.01	32.52±2.71	186.17±15.43
CLP+LR12	55.15±3.72 [△]	24.48±1.22 [△]	163.35±13.08 [△]
CLP+DNase I	48.02±4.35 [△]	18.20±1.07 [△]	147.91±16.13 [△]

*P<0.01 compared with sham operation group; [△]P<0.01 compared with model group.

降低 ($P<0.01$), CLP+LR12-scr组小鼠心肌组织中MPO-NDA和NE-DNA水平差异无统计学意义 ($P>0.05$)。见表4。

2.6 各组小鼠心肌组织中Ly6G和cit-H3蛋白共表达情况 与假手术组比较,模型组小鼠心肌组织中Ly6G和cit-H3蛋白共表达增加;与模型组比较,CLP+LR12组和CLP+DNase I组小鼠心肌组织中Ly6G和cit-H3蛋白共表达减少,CLP+LR12-scr组

表4 各组小鼠心肌组织中MPO-DNA和NE-DNA水平
Tab.4 Levels of MPO-DNA and NE-DNA in myocardium tissue of mice in various groups (n=8, $\bar{x}\pm s$)

Group	MPO-DNA[$w_B/(\mu\text{g}\cdot\text{g}^{-1})$]	NE-DNA[$w_B/(\text{ng}\cdot\text{g}^{-1})$]
Sham operation	10.32±1.15	7.88±1.36
Model	31.89±4.04*	27.53±2.28*
CLP+LR12-scr	30.42±3.85	25.76±3.19
CLP+LR12	22.98±2.72 [△]	17.60±1.88 [△]
CLP+DNase I	19.36±2.24 [△]	15.87±1.45 [△]

*P<0.01 compared with sham operation group; [△]P<0.01 compared with model group.

小鼠心肌组织中Ly6G和cit-H3蛋白共表达无明显变化。见图2。

2.7 各组小鼠心肌组织中TREM-1、MPO、NE和cit-H3蛋白表达水平 与假手术组比较,模型组小鼠心肌组织中TREM-1、MPO、NE和cit-H3蛋白表达水平均明显升高 ($P<0.01$)。与模型组比较,CLP+LR12组小鼠心肌组织中TREM-1、MPO、NE和cit-H3蛋白表达水平均明显降低 ($P<0.01$);

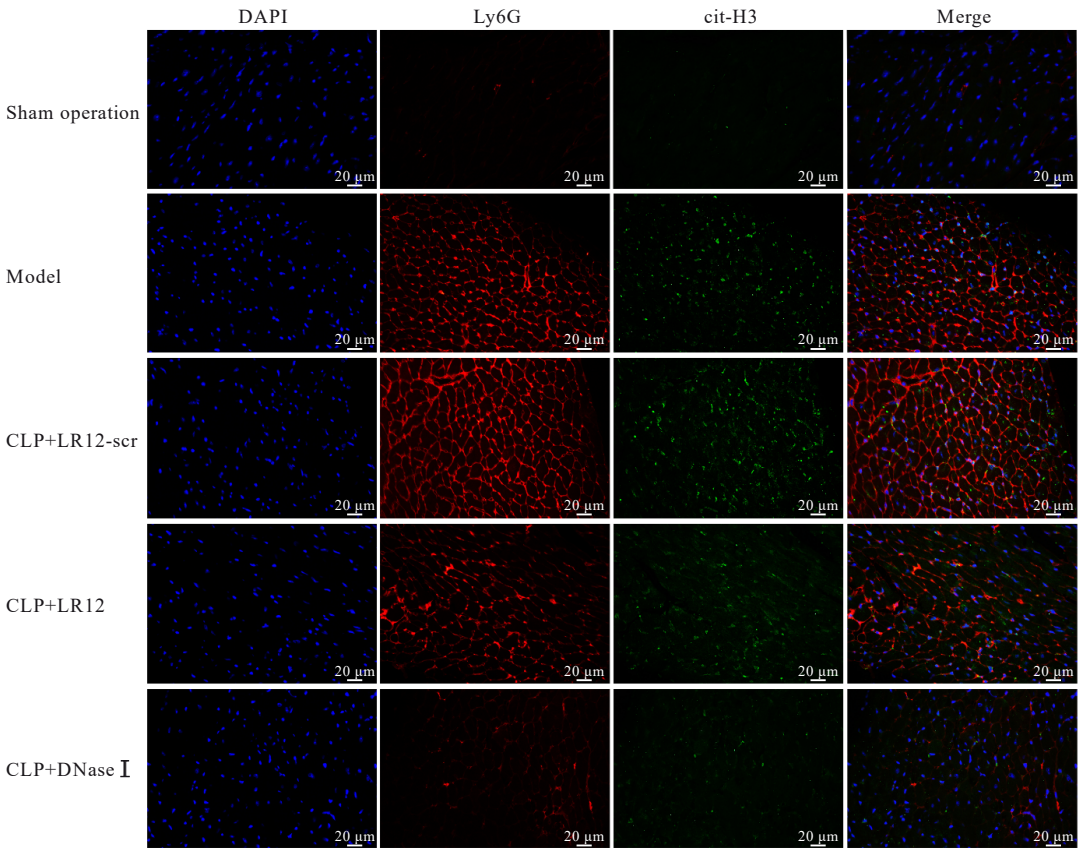
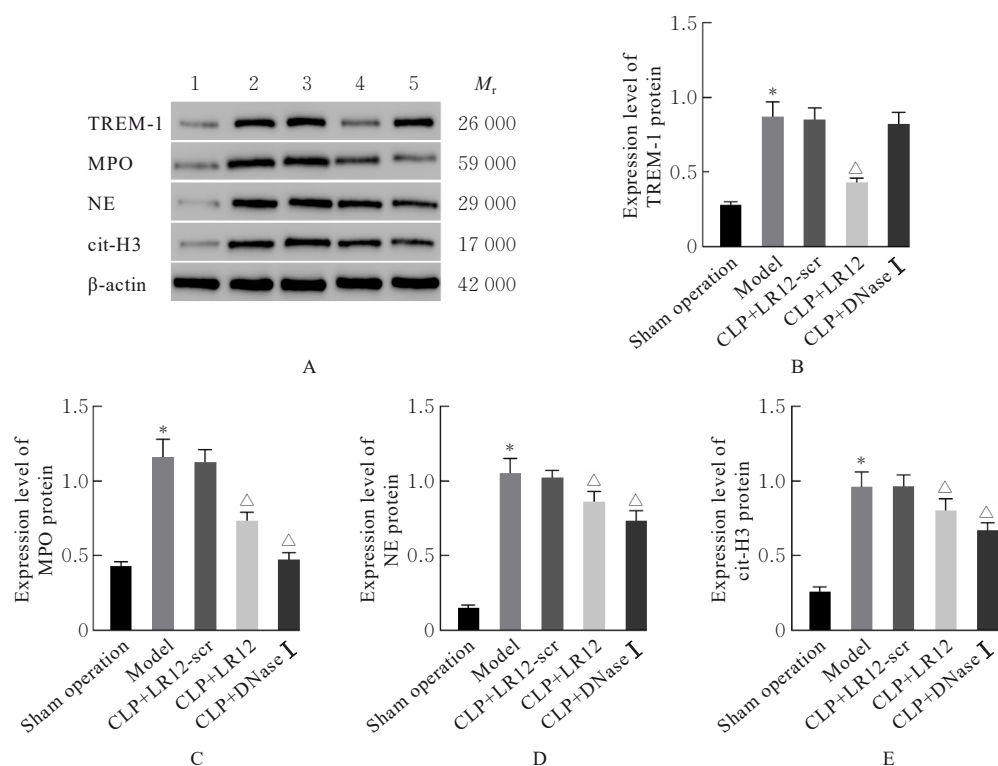


图2 各组小鼠心肌组织中Ly6G和cit-H3蛋白共表达情况(免疫荧光染色)

Fig.2 Co-expression of Ly6G and cit-H3 proteins in myocardium tissue of mice in various groups (Immunofluorescence)

CLP+DNase I 组小鼠心肌组织中 MPO、NE 和 cit-H3 蛋白表达水平均明显降低 ($P<0.01$), TREM-1 蛋白表达水平无明显变化 ($P>0.05$);

CLP+LR12-scr 组小鼠心肌组织中各蛋白表达水平均无明显变化, 差异无统计学意义 ($P>0.05$)。见图 3。



A: Electrophoregram (Lane 1: Sham operation group; Lane 2: Model group; Lane 3: CLP+LR12-scr group; Lane 4: CLP+LR12 group; Lane 5: CLP+DNase I group); B: TREM-1; C: MPO; D: NE; E: Cit-H3. * $P<0.01$ compared with sham operation group; $\Delta P<0.01$ compared with model group.

图3 各组小鼠心肌组织中 TREM-1、MPO、NE 和 cit-H3 蛋白表达电泳图(A)及直条图(B~E)

Fig. 3 Electrophoregram(A) and histograms(B-E) of expressions of TREM-1, MPO, NE and cit-H3 proteins in myocardium tissue of mice in various groups

3 讨论

脓毒症心肌损伤是由脓毒症引发的心脏功能障碍, 其机制涉及过度炎症、氧化应激、线粒体损伤和细胞死亡等, 严重时可导致心功能衰竭甚至死亡^[11-12]。目前临床治疗以抗感染和支持治疗为主, 尚缺乏特异性治疗药物。CK-MB 和 cTnI 是经典的心肌损伤标志物, 其血清水平可反映心肌损伤程度^[13]。本研究通过 CLP 手术构建脓毒症小鼠模型, 结果显示: 模型组小鼠表现为精神萎靡, 心肌组织结构紊乱; 与假手术组比较, 模型组小鼠 LVEF 和 LVFS 明显降低, 血清中 CK-MB 和 cTnI 水平明显升高, 提示小鼠心脏功能受损, 表明脓毒症心肌损伤模型构建成功。

TREM-1 是免疫球蛋白超家族中的一种活化性受体, 主要表达于髓系细胞表面^[14]。该受体可在

中性粒细胞和单核细胞表面受诱导而高水平表达, 通过自然杀伤激活受体相关蛋白 12 (DNAX-associated protein 12, DAP12) 衔接蛋白的介导, 增强 Toll 样受体 (Toll-like receptors, TLRs) 对微生物侵袭的应答, 促进细菌和真菌感染时促炎细胞因子的分泌^[15-16]。鉴于 TREM-1 在炎症放大过程中的关键作用, 其被认为是脓毒症天然免疫应答的关键调控因子。LR12 作为 TREM-1 特异性抑制肽, 可通过选择性阻断 TREM-1 受体活化, 在脓毒症动物模型中有效抑制炎症级联反应, 改善器官功能, 显示出重要的治疗潜力。YANG 等^[17] 研究显示: 在脓毒症小鼠心肌组织和经脂多糖 (lipopolysaccharide, LPS) 刺激的心肌细胞 HL-1 中, TREM-1 表达均明显上调, 表明 TREM-1 激活可诱导心肌细胞焦亡, 导致脓毒症心肌病发生;

LR12干预可抑制细胞焦亡,改善脓毒症小鼠的心脏功能并延长其存活时间。谭子富等^[18]研究显示:LR12可通过调控TLR4/核因子 κ B(nuclear factor- κ B, NF- κ B)信号通路减轻心肌炎症,对心肌缺血再灌注损伤大鼠的心肌组织具有保护作用。本研究结果显示:经LR12干预后,CLP+LR12组小鼠精神状态好转,心肌组织病理损伤明显改善,心功能指标LVEF和LVFS明显升高,且血清中CK-MB和cTnI水平下调;LR12-scr干预后,CLP+LR12-scr组小鼠心功能无明显改善,表明LR12可有效改善脓毒症小鼠心肌损伤,对心肌组织具有保护作用。

中性粒细胞是机体抵抗细菌感染的核心免疫细胞,然而其过度激活可通过多种病理途径加剧组织损伤,甚至诱发器官功能衰竭^[19-20]。研究^[21-23]表明:NETs是中性粒细胞重要的抗菌效应机制,其形成失调会明显加重脓毒症相关组织损伤。研究^[24-25]显示:MPO和NE是NETs的主要组分,MPO-DNA及NE-DNA复合物水平与NETs形成水平呈正相关关系。cit-H3是NETs形成的特异性分子标志,Ly6G是中性粒细胞高表达的表面标志物,两者共定位可精准识别中性粒细胞来源的NETs结构,为NETs的体内形成提供直接证据^[26]。GAO等^[27]研究显示:红景天苷可通过调控丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)信号通路,抑制NETs形成,减轻脓毒症诱导的肺损伤。SHEN等^[28]研究发现:抑制补体成分5a受体1(complement component 5a receptor 1, C5aR1)水平可通过调控NETs的过度表达来平衡脓毒症大鼠体内炎症水平,改善脓毒症引发的多器官损伤。上述研究提示:精确调控NETs的动态平衡可能是改善脓毒症炎症反应和组织损伤的关键干预策略。本研究结果显示:经NETs直接抑制剂DNase I^[29]干预后,CLP+DNase I组小鼠心肌损伤明显好转,证实了NETs在脓毒症病程中的作用。BOUFENZER等^[6]研究显示:TREM-1可通过增强TLR信号通路促进中性粒细胞释放NETs,而TREM-1抑制肽LR12可减少NETs生成,改善脓毒症中NETs介导的血管功能障碍,提示TREM-1对NETs形成过程具有调控作用。本研究结果显示:模型组小鼠血清炎症因子水平升高,心肌组织中Ly6G和cit-H3蛋白共表达增加,MPO-DNA和NE-DNA水平均明显升高,提示脓毒症小鼠存

在明显的炎症反应激活和NETs过度形成;LR12干预后,CLP+LR12组小鼠上述异常指标均得到改善,提示LR12可能通过下调NETs水平来减轻过度炎症反应和心肌损伤。上述研究结果表明:LR12可明显降低TREM-1表达及NETs相关蛋白MPO、NE和cit-H3水平,而DNase I不影响TREM-1表达,说明LR12可能通过抑制TREM-1受体,减少NETs形成,进而发挥心肌保护作用。

综上所述,TREM-1抑制肽LR12可降低小鼠体内炎症水平,减轻脓毒症心肌损伤,改善心功能,其作用机制可能与下调TREM-1表达和抑制NETs形成有关联。本研究揭示了TREM-1作为脓毒症心肌损伤治疗靶点的潜在价值。但当前研究结论主要基于动物模型获得,未来计划通过体外细胞实验进一步验证LR12的作用机制,并探讨其可能存在的其他作用途径。

利益冲突声明:

所有作者声明不存在利益冲突。

作者贡献声明:

李金玉参与研究设计、实验操作和论文撰写及修改,李忠辉、程爱斌和部璇参与实验操作及数据采集,白静参与数据整理和分析,王建军参与研究设计和论文审阅。

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