论著·基础医学

左旋肉碱对脓毒症心肌收缩功能障碍的改善作用及机制

王志华[1],周远群[2],向鑫明[2],张千威[2],欧阳杏楠[2],张 杰[2],李 涛[2],田林强[1],刘良明[2] [1]新乡医学院第三附属医院外科,河南新乡;[2]陆军军医大学大坪医院战伤休克与输血研究室,创伤与化学中毒全国重点实验室,重庆

[摘要] **目的** 探讨左旋肉碱(L-carnitine)对脓毒症心肌收缩功能障碍的改善作用及其机制。**方法** 采用盲肠结扎穿孔术(cecal ligation and puncture,CLP)建立小鼠脓毒症模型,将10周龄SPF级雄性C57BL/6小鼠(体质量20~30 g)按照随机数字表法分为5组:假手术(Sham)组,脓毒症(Sepsis)组,左旋肉碱(L-carnitine)组,左旋肉碱治疗+依托莫司[L-carnitine+Etomoxir(Eto)]组,Eto组。利用超声心动图检测小鼠心功能,ELISA方法检测血清中肌酸激酶同工酶MB(creatine kinase-MB isoenzyme, CK-MB)水平,记录小鼠72 h存活情况,以评估L-carnitine对心脏功能的影响。分离心肌细胞,利用细胞微张力仪检测心肌细胞收缩功能与钙瞬变。取各组小鼠心肌组织,采用ELISA方法检测甘油三酯(triglyceride, TG)、游离脂肪酸(free fatty acid, FFA)和三磷酸腺苷(adenosine triphosphate, ATP)含量。采用脂多糖(lipopolysaccharide,LPS)刺激HL-1心肌细胞12 h,构建脓毒症体外模型,分为正常(CTRL)组、LPS组、左旋肉碱(L-carnitine)组、左旋肉碱治疗+依托莫司(L-carnitine+Eto)组和依托莫司(Eto)组。采用ELISA检测心肌细胞中TG、FFA和ATP含量以及肉碱棕榈酰转移酶1A(carnitine palmitoyltransferase 1A,CPT1A)的活性,利用细胞能量代谢分析系统检测脂肪酸氧化能力,采用Western blot检测心肌细胞CPT1A的蛋白表达;采用BODIPY-FL-C16(绿色荧光标记的棕榈酸)孵育细胞,利用激光共聚焦显微镜观察棕榈酸在细胞质和线粒体的分布。**结果** 与Sham组比较,Sepsis组小鼠心功能明显受损,表现为射血分数、平均动脉血压降低($P<0.05$),且心损标志物CK-MB水平升高($P<0.05$),L-carnitine处理显著改善脓毒症小鼠心肌功能并恢复血压,使脓毒症小鼠的存活率从12.50%提升至81.25%($P<0.05$)。与Sham组比较,Sepsis组急性分离的单个心肌细胞的收缩功能和钙瞬变显著下降($P<0.05$),L-carnitine治疗可显著恢复脓毒症心肌细胞的收缩功能和钙释放能力($P<0.05$)。体内外实验均发现,Sepsis组和LPS组TG和FFA含量升高($P<0.05$),ATP含量降低($P<0.05$),L-carnitine处理可显著逆转此现象。LPS组心肌细胞对脂肪酸的基础氧化速率、最大氧化能力较CTRL组显著降低($P<0.05$),L-carnitine治疗可明显改善上述指标。与CTRL组比较,LPS组心肌细胞中CPT1A的表达和活性明显降低($P<0.05$),L-carnitine处理明显增加CPT1A的表达和活性($P<0.05$)。LPS组心肌细胞中绿色荧光标记的棕榈酸主要在细胞质形成大量颗粒或团块状聚集,且与线粒体共定位极少,L-carnitine组心肌细胞胞质的绿色荧光颗粒较小,且与线粒体共定位增加,而L-carnitine+Eto组与LPS组呈相似的现象。此外,体内外实验均发现,CPT1A抑制剂Eto处理逆转了L-carnitine的作用,L-carnitine+Eto组中ATP的含量较L-carnitine组显著降低($P<0.05$),而FFA含量显著增加($P<0.05$)。**结论** 左旋肉碱通过CPT1A依赖性机制促进脂肪酸进入线粒体进行β-氧化,从而改善脓毒症心肌细胞脂肪酸氧化功能障碍,进一步改善脓毒症心肌细胞收缩功能。

[关键词] 脓毒症;心肌收缩功能障碍;脂肪酸氧化;左旋肉碱;肉碱棕榈酰转移酶1A

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[通信作者] 刘良明,E-mail:lmliu62@tmmu.edu.cn

田林强,E-mail:wstiansenzhi@126.com

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Effect and underlying mechanism of L-carnitine improving myocardial systolic dysfunction in sepsis mice

WANG Zhihua[1], ZHOU Yuanqun[2], XIANG Xinming[2], ZHANG Qianwei[2], OUYANG Xingnan[2], ZHANG Jie[2], LI Tao[2], TIAN Linqiang[1], LIU Liangming[2] [1]Department of Surgery, the Third Affiliated Hospital of Xinxiang Medical University, Xinxiang, Henan; [2]National Key Laboratory of Trauma and Chemical Poisoning, Research Laboratory of War Trauma, Shock and Blood Transfusion, Daping Hospital, Army Medical University, Chongqing, China

[**Abstract**] Objective To explore the protective effect of L-carnitine on myocardial systolic dysfunction in sepsis and its underlying mechanism. Methods A mouse sepsis model was established by cecal ligation and puncture (CLP). Ten-week-old male SPF-grade C57BL/6 mice (body weight 20~30 g) were randomly divided into 5 groups *via* random number table: Sham group, Sepsis group, L-carnitine group, L-carnitine+Etomoxir (Eto) group, and Eto group. Echocardiography assessed cardiac function, ELISA measured serum creatine kinase isoenzyme MB (CK-MB) levels, and 72-hour survival rates were recorded to evaluate L-carnitine's effects on cardiac function. Cardiomyocytes were isolated, and a cell microtensiometer was used to detect cardiomyocyte contractile function and calcium transients. Myocardial tissues were collected from each group, and ELISA was used to determine the contents of triglyceride (TG), free fatty acid (FFA), and adenosine triphosphate (ATP). An *in vitro* sepsis model was constructed by stimulating HL-1 cardiomyocytes with lipopolysaccharide (LPS) for 12 hours, which was divided into 5 groups: control (CTRL) group, LPS group, L-carnitine group, L-carnitine + Eto group, and Eto group. ELISA was used to detect the contents of TG, FFA, and ATP as well as the activity of carnitine palmitoyltransferase 1A (CPT1A) in cardiomyocytes. A cellular energy metabolism analysis system was employed to measure fatty acid oxidation capacity, and Western blot was used to detect the protein expression of CPT1A in cardiomyocytes. BODIPY-FL-C16 (green fluorescently labeled palmitic acid) was utilized to detect the distribution of fatty acids in the cytoplasm and mitochondria *via* immunofluorescence technology, thereby observing the ability of cells to transport fatty acids into mitochondria. Results Compared with the Sham group, cardiac function was significantly impaired in the Sepsis group, as evidenced by decreased ejection fraction and mean arterial pressure ($P<0.05$), along with elevated levels of the cardiac injury marker CK-MB ($P<0.05$). Treatment with L-carnitine significantly improved myocardial function, restored blood pressure in septic mice, and increased their survival rate from 12.50% to 81.25% ($P<0.05$). Compared with the Sham group, the contractile function and calcium transients of acutely isolated single cardiomyocytes were significantly reduced in the Sepsis group ($P<0.05$), while L-carnitine treatment remarkably restored the contractile function and calcium release capacity of septic cardiomyocytes ($P<0.05$). Both *in vivo* and *in vitro* experiments showed that TG and FFA levels were significantly increased ($P<0.05$), and ATP levels was significantly decreased ($P<0.05$) in the Sepsis and LPS groups—effects significantly reversed by L-carnitine treatment. Compared with the CTRL group, the basal oxidation rate and maximum oxidation capacity of fatty acids in cardiomyocytes of the LPS group were significantly reduced ($P<0.05$), and L-carnitine treatment notably improved these indicators. Compared with the CTRL group, the expression and activity of CPT1A in cardiomyocytes of the LPS group were significantly decreased ($P<0.05$), while L-carnitine treatment significantly increased the expression and activity of CPT1A ($P<0.05$). In LPS group cardiomyocytes, green fluorescently labeled palmitic acid primarily formed numerous granular/clumpy aggregates in the cytoplasm with minimal mitochondrial colocalization. In the L-carnitine group, the green fluorescent granules in the cytoplasm of cardiomyocytes were smaller, and colocalization with mitochondria was increased. However, the L-carnitine + Eto group exhibited similar phenomena to the LPS group. In addition, both *in vivo* and *in vitro* experiments demonstrated that treatment with the CPT1A inhibitor Eto reversed the effect of L-carnitine. Compared with the L-carnitine group, the ATP content in the L-carnitine + Eto group was significantly decreased ($P<0.05$), while the FFA content was significantly increased ($P<0.05$). Conclusion L-carnitine facilitates fatty acid entry into mitochondria for β-oxidation *via* a CPT1A-dependent mechanism, thereby ameliorating fatty acid oxidation dysfunction in septic cardiomyocytes and improving myocardial contractile function.

[**Key words**] sepsis; myocardial contractile dysfunction; fatty acid oxidation; L-carnitine; carnitine palmitoyltransferase 1A

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脓毒症是因宿主对感染失调反应引起的危及生命的器官功能障碍[1],是重症监护病房的主要死亡原因。脓毒症所致的心脏功能障碍是脓毒症患者最严重的并发症,其病死率高达70%~90%[2]。心肌收缩功能障碍是脓毒症相关心肌功能障碍(sepsis-associated myocardial dysfunction,SIMD)的核心特征和影响预后的关键因素,表现为心室射血分数降低、每搏输出量下降等[3]。目前,针对SIMD的治疗,临床上主要采取液体复苏、血管升压药物及正性肌力药物等支持性手段,缺乏特异、高效的靶向治疗方案,尤其是针对其核心的收缩功能障碍[4]。因此,进一步探索具有潜在治疗作用的药物具有重要临床意义。

研究表明,脓毒症时,全身性炎症风暴、线粒体功能障碍、能量代谢紊乱以及钙离子稳态失调等多重因素共同作用,损害患者心肌细胞的收缩能力[5]。其中,心肌细胞能量代谢障碍,特别是脂肪酸氧化(fatty acid oxidation,FAO)这一主要能量供应途径的显著受损,被认为是导致心肌收缩力下降的关键环节之一[6],靶向FAO可能是治疗脓毒症导致的心肌收缩功能障碍的有效途径。

左旋肉碱(L-carnitine)是人体内天然存在的一种类氨基酸衍生物[7],其主要生理功能是形成酰基肉碱,将活化的长链脂肪酸从细胞质转运至线粒体基质,促进脂肪酸β氧化产生ATP[8]。此外,L-carnitine还具有稳定细胞膜结构、清除氧自由基、减轻氧化应激损伤、调节钙离子平衡等多重生物学效应[9]。基于其在能量代谢和细胞保护中的核心作用,L-carnitine在多种疾病(如心肌缺血、心力衰竭)中显示出潜在的器官功能保护功效。有研究发现,L-carnitine能减轻脂多糖(lipopolysaccharide,LPS)诱导的脓毒症小鼠急性肾损伤和炎症[10],但L-carnitine是否能改善脓毒症心肌收缩功能,尚未见报道。

因此,本研究通过盲肠结扎穿孔术(cecal ligation and puncture,CLP)在动物水平建立脓毒症小鼠模型,并在细胞水平以LPS处理模拟脓毒症,观察L-carnitine对脓毒症心肌收缩功能和钙瞬变的改善作用,研究其对脓毒症心肌细胞脂肪酸氧化能力的调节作用,旨在初步明确L-carnitine对脓毒症心肌收缩功能的保护作用及机制。

1 材料与方法

1.1 实验动物

雄性10周龄C57BL/6小鼠(体质量20~30 g)由陆军特色医学中心动物实验中心提供。该研究已获陆军军医大学实验动物福利伦理审查委员会批准(AMUWEC20247100),且所有操作符合动物伦理要求。

1.2 药物与试剂

常规化学试剂均为分析纯;Ⅱ型胶原酶购自美国Sigma公司;Fura-2-AM购自美国Invitrogen公司;肉碱棕榈酰转移酶1A(carnitine palmitoyltransferase 1A,CPT1A)活性检测试剂盒购自索莱宝公司;BSA、BODIPY-FL-C16(BODIPY-FL荧光染料标记的十六碳棕榈酸)、Amplex Red游离脂肪酸检测试剂盒、Amplex Red甘油三酯检测试剂盒、ATP检测试剂盒购自上海碧云天生物技术有限公司;依托莫司(etomoxir,Eto)和L-carnitine购自美国MCE公司;小鼠肌酸激酶同工酶MB(creatine kinase-MB isoenzyme, CK-MB)酶联免疫吸附测定试剂盒购自武汉伊莱瑞特生物科技股份有限公司;XF棕榈酸氧化压力测试试剂盒购自安捷伦科技公司;CPT1A抗体购自美国Affinity Biosciences有限公司;HL-1心肌细胞购自浙江美森细胞科技有限公司。

1.3 溶液配制

无钙台氏液:NaCl 137 mmol/L、KCl 5.4 mmol/L、NaH_2PO_4 1.2 mmol/L、$MgCl_2$ 1.2 mmol/L、HEPES 3.3 mmol/L、Glucose 11 mmol/L,NaOH调节pH至7.3~7.4;含钙(1.8 mmol/L)台氏液:在无钙台式液中加入$CaCl_2$ 1.8 mmol/L,NaOH调节pH至7.3~7.4;KB液:L-Glutamic acid 120 mmol/L、KOH 120 mmol/L、KCl 110 mmol/L、KH_2PO_4 10 mmol/L、Taurine 10 mmol/L、$MgSO_4\cdot 7H_2O$ 1.8 mmol/L、K-EGTA 0.5 mmol/L、HEPES 10 mmol/L、Glucose 20 mmol/L、BSA 0.2%,KOH调节pH=7.2;消化液的配制:无钙台氏液40 mL、Ⅱ型胶原酶24 mg、BSA 40 mg。

1.4 脓毒症模型制备

C57BL/6小鼠术前禁食12 h,自由饮水,按照本实验室前期研究的方法[11],采用CLP制备小鼠脓毒症模型。为确保模型的一致性,建模过程按照标准化操作流程进行,所有关键操作由同一名熟练实验者完成,并严格控制以下条件:①盲肠结扎位置统一为距盲肠末端1/2处;②穿孔针规格统一为22G无菌针头,穿孔数量固定为1孔;③术后均给予1 mL无菌生理盐水腹腔注射以补充体液,避免脱水对模型稳定性的影响。假手术组(仅开腹后轻柔翻动盲肠,不进行结扎和穿孔,其余操作与CLP组一致)。

1.5 实验分组及设计

将90只C57BL/6小鼠按照随机数字表法分为3组(n=30):Sham组,Sepsis组,L-carnitine组。其中,Sepsis组小鼠采用盲肠结扎穿孔术制作脓毒症模型;L-carnitine组于术后1 h腹腔注射500 mg/kg[12] L-carnitine。CLP术后24 h,采用超声心动图(中国柏

塔科技深圳有限公司)检测小鼠心脏功能并经颈动脉插管监测血压(n=8),随后取眼球血用ELISA检测小鼠血清中CK-MB水平(n=4);取小鼠心脏,用Langendorff灌流系统分离心肌细胞,检测心肌收缩力和钙敏感性(n=8,每只动物检测多个细胞);取心肌组织测量甘油三酯(triglyceride, TG)、游离脂肪酸(free fatty acid, FFA)和三磷酸腺苷(adenosine triphosphate, ATP)含量(n=6);存活实验中,观察各组小鼠CLP术后72 h存活情况(n=16)。

将40只C57BL/6小鼠按照随机数字表法分为5组(n=8):Sham组,Sepsis组,L-carnitine组,L-carnitine+Eto组和Eto组。其中,L-carnitine组于术后1 h腹腔注射500 mg/kg L-carnitine;L-carnitine+Eto组于术后1 h分别腹腔注射500 mg/kg L-carnitine和20 mg/kg Eto;Eto组于术后1 h腹腔注射20 mg/kg Eto;CLP术后24 h,检测小鼠心肌组织中ATP含量及FFA水平(n=8)。

1.6 超声心动图评估小鼠左心室功能

用0.3%戊巴比妥钠腹腔麻醉小鼠,固定、剃掉小鼠胸前毛发,使用便携式多普勒超声系统评估小鼠的左心室功能。将仪器切到左室长轴,在二尖瓣腱索处使用M超记录图像,选取任意3个连续的心动周期,测量左心室舒张末期内径(left ventricular end-diastolic diameter, LVEDD)、左心室收缩末期内径(left ventricular end-systolic diameter, LVESD)、左心室舒张末容积(left ventricular end-diastolic volume, LVEDV),左心室收缩末容积(left ventricular end-systolic volume, LVESV),根据公式计算小鼠左心室射血分数(left ventricular ejection fraction, LVEF)。公式为:LVEF=[(LVEDV−LVESV)/LVEDV]×100%。

1.7 检测小鼠血清中CK-MB水平

取小鼠眼眶取血,室温静置15 min,以4 ℃、3 000 r/min离心10 min,取上清,按照小鼠CK-MB酶联免疫吸附测定试剂盒使用说明书检测小鼠血清中CK-MB水平。

1.8 小鼠存活情况观察

小鼠建立脓毒症模型后,缝合伤口,放回笼中饲养,禁食,自由饮水。每小时观察1次,总计观察72 h,记录小鼠的状态和存活时间。

1.9 心肌细胞收缩力及钙瞬变的测定

按照本实验室前期研究的方法[13],采用Langendorff灌流系统制备单个心肌细胞,并用浓度梯度法复钙,最后将细胞重悬于1 mL含钙台式液中。将上述复钙后的心肌细胞用Fura-2-AM染料(2 μmol/L)在37 ℃下染色0.5 h(避光)。在避光条件下,取50 μL上述心肌细胞悬液于细胞微张力仪(美国IonOptix LLC)的刺激浴槽中,加入含钙台氏液至淹没刺激电极。镜下找到收缩正常,肌节较清晰的单个心肌细胞,置于视野中央,调整光圈大小至孔径只包含一个心肌细胞,以减少运动伪影的干扰。在15 V电压,1 HZ频率的条件下刺激心肌细胞,并用340 nm与380 nm波长的紫外激发光分别照射细胞,在480~520 nm处检测对应的荧光强度。每4毫秒记录1次,记录5 min内心肌细胞的肌节长度变化曲线和钙瞬变曲线。以心肌细胞肌节收缩幅度反映收缩能力,收缩幅度的计算方法:收缩幅度=(静息肌节长度−收缩末肌节长度)/静息肌节长度。肌节长度标准化为每组静息肌节长度平均化,其余肌节长度除以其平均值。以340 nm的荧光强度与380 nm的荧光强度的比值变化反映细胞内钙离子浓度变化,即荧光强度F340/F380表示心肌细胞内钙瞬变。心肌细胞收缩钙瞬变幅度=ΔF(收缩末荧光强度$_{F340/F380}$−静息荧光强度$_{F340/F380}$)/F0(静息荧光强度$_{F340/F380}$)。

1.10 TG、FFA和ATP含量检测及CPT1A活性检测

取小鼠心肌组织,分别按Amplex Red甘油三酯检测试剂盒、Amplex Red游离脂肪酸检测试剂盒和ATP检测试剂盒说明书测定小鼠心脏组织中TG、FFA及ATP含量。

HL-1心肌细胞接种于T25培养瓶,细胞融合度达60%时进行加药处理,分为:CTRL组;LPS组:10 μg/mL LPS刺激12 h;L-carnitine组:10 μg/mL LPS刺激后,给予L-carnitine(100 μmol/L)处理12 h;L-carnitine+Eto组:10 μg/mL LPS刺激后,分别给予L-carnitine(100 μmol/L)和Eto(20 μmol/L)处理12 h;Eto组:10 μg/mL LPS刺激后,给予Eto(20 μmol/L)处理12 h。按Amplex Red甘油三酯检测试剂盒、Amplex Red游离脂肪酸检测试剂盒、ATP检测试剂盒说明书和CPT1A活性检测试剂盒测定心肌细胞中TG、FFA、ATP含量和CPT1A活性。

1.11 细胞能量代谢分析系统检测脂肪酸氧化能力

接种HL-1心肌细胞,当细胞融合度达60%~75%时,进行加药处理,分组及处理方式同1.10,按照XF棕榈酸氧化压力试剂盒说明书检测脂肪酸氧化压力。上机前一天,将细胞生长培养基更换为底物限制生长培养基(含0.5 mmol/L葡萄糖、1 mmol/L谷氨酰胺、1%胎牛血清和0.5 mmol/L L-carnitine)。水化探针板放至37 ℃无二氧化碳细胞培养箱中水化过夜。上机当天,配置检测液(含2 mmol/L葡萄糖和0.5 mmol/L L-carnitine的Seahorse XF DMEM培养基)。细胞用检测液洗2次后每孔加入415 μL检测液,放入37 ℃无二氧化碳细胞培养箱中脱气60 min,等待上机。同时,探针板加药孔中分别加入Etomoxir(A孔)、寡霉素(B孔)、FCCP(C孔)、鱼藤酮/抗霉素A(D孔),上机校准。校

准完成后，从37 ℃无二氧化碳细胞培养箱中取出细胞培养板，每孔加入85 μL 1×BSA或1×棕榈酸-BSA复合物（Palmitate-BSA），上机检测，使用安捷伦Seahorse Analytics软件进行数据分析，自动计算棕榈酸氧化压力测试参数：基础呼吸、对抑制剂的急性响应和最大呼吸。

1.12　Western blot法检测CPT1A蛋白在心肌细胞中的表达

HL-1心肌细胞分组为CTRL、LPS、L-carnitine组。收取细胞，加入RIPA裂解液冰上裂解30 min，12 000×*g*、4 ℃离心20 min，取上清后加上样缓冲液，100 ℃煮6 min使蛋白变性。蛋白用10%SDS-PAGE凝胶电泳后转至PVDF膜，将PVDF膜用5%脱脂奶粉常温封闭2 h，TBST稀释的CPT1A一抗（1∶1 000）和GAPDH一抗（1∶5 000）4 ℃孵育过夜，最后用TBST清洗后孵育二抗（1∶20 000）1 h。用双色红外成像激光系统扫描，获得CPT1A的蛋白电泳图像。

1.13　免疫荧光检测

HL-1心肌细胞提前一天接种在共聚焦皿中，当密度为70%~80%时，进行加药处理，分组及处理方式同1.10。用BODIPY-FL-C16（BODIPY FL荧光染料标记的十六碳棕榈酸）孵育心肌细胞（浓度为1∶1 000）孵育20 min，培养基清洗2次，然后用PK mito orange染线粒体（浓度为1∶5 000）孵育15 min，再用PBS清洗2次，共聚焦显微镜下观察。用Image J软件分析棕榈酸和线粒体的共定位。

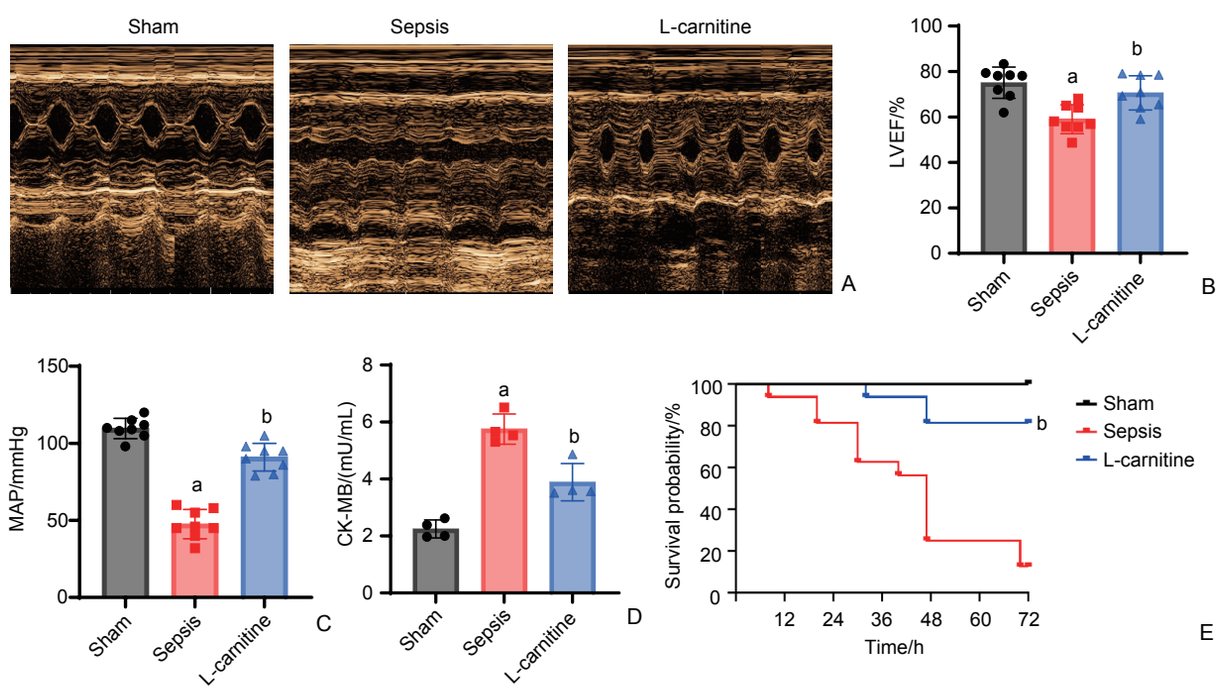
1.14　统计学分析

采用SPSS 26.0统计软件分析。数据均以$\bar{x}\pm s$表示，多组间比较采用单因素方差分析，涉及多因素时的组间比较采用双因素方差分析，生存分析采用Kaplan-Meier法绘制生存曲线，组间比较采用Log-rank检验。$P<0.05$为差异有统计学意义。

2　结果

2.1　L-carnitine对脓毒症心肌功能的改善作用

为了观察L-carnitine对脓毒症诱导的心肌损伤的改善作用，CLP术后1 h给予L-carnitine处理，术后24 h观察各组小鼠的心肌功能指标。超声心动图结果显示，与Sham组相比，Sepsis组小鼠左心室射血分数明显降低，L-carnitine可以明显恢复脓毒症小鼠的射血分数（$P<0.05$，图1A、B）。Sepsis组小鼠平均动脉压也明显降低，仅（47.63±9.52）mmHg，L-carnitine组血压明显改善，回升至（91.00±9.00）mmHg（$P<0.05$，图1C）。同时，脓毒症小鼠血清中CK-MB水平较Sham组显著升高，L-carnitine处理明显降低CK-MB水平，下降了



A：Echocardiographic assessment of cardiac function in mice；B：Statistical analysis of echocardiographic parameters (left ventricular ejection fraction，LVEF) in mice (*n*=8)；C：Mean arterial pressure (MAP) measurements in mice；D：Serum CK-MB levels in mice measured by ELISA (*n*=4)；E：Survival curve of septic mice (*n*=16). a：$P<0.05$，compared with the Sham；b：$P<0.05$，compared with the Sepsis

图1　L-carnitine对脓毒症心肌功能的改善作用（$\bar{x}\pm s$）

Figure 1　Improvement of L-carnitine on myocardial function in sepsis（$\bar{x}\pm s$）

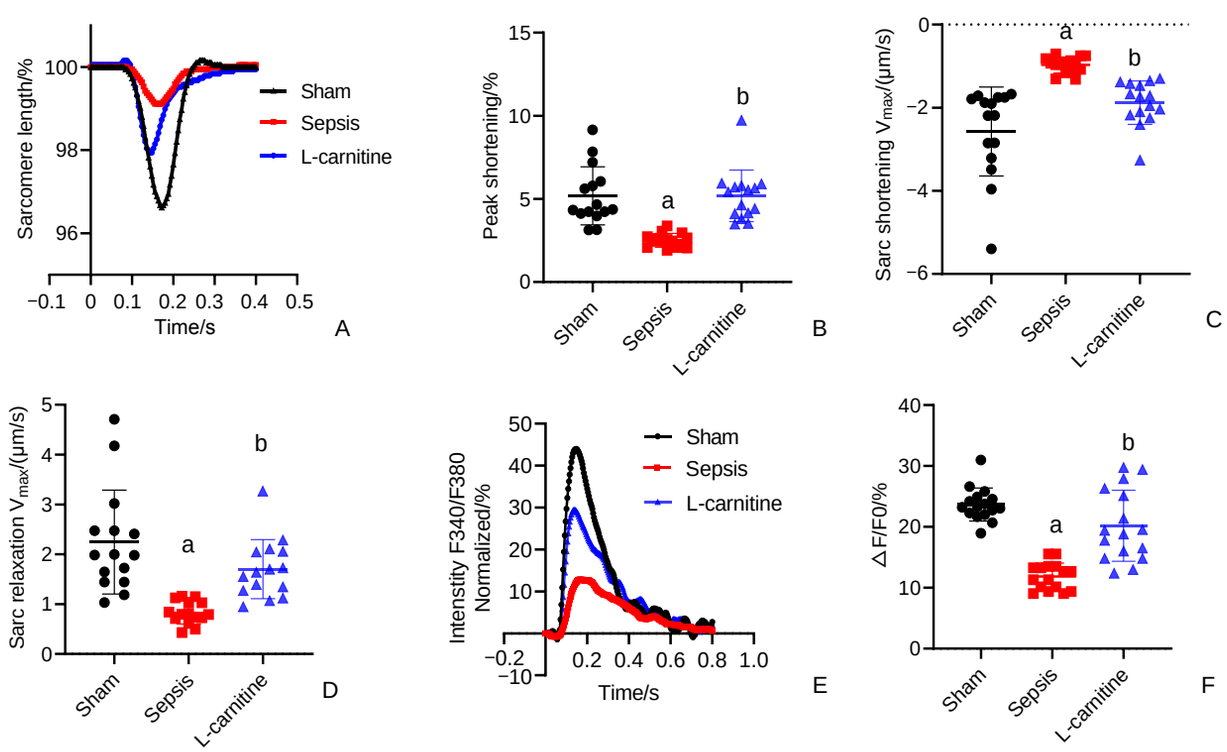
32.34%(P<0.05,图1D)。动物存活实验发现,CLP术后72 h,Sepsis组小鼠的存活率仅为12.50%(2/16),L-carnitine处理提高脓毒症小鼠存活率至81.25%(13/16,P<0.05,图1E)。这些结果表明,L-carnitine能明显改善脓毒症诱导的心肌功能障碍。

2.2 L-carnitine对脓毒症小鼠心肌细胞收缩功能和钙瞬变的改善作用

为观察L-carnitine对脓毒症小鼠心肌细胞收缩功能的改善作用,造模24 h后,取各组小鼠心肌组织急性分离心肌细胞,利用细胞微张力仪观察单个心肌细胞收缩功能的变化。结果发现,与Sham组比较,Sepsis组小鼠的心肌细胞收缩幅度显著下降,降幅达51.61%(P<0.05);而给予L-carnitine治疗后,小鼠心肌细胞的收缩幅度较Sepsis组显著增加(P<0.05,图2A、B)。同时观察到,Sepsis组小鼠心肌细胞的最大收缩速度和最大舒张速度也显著减慢(P<0.05);L-carnitine治疗组的最大收缩速度和最大舒张速度则较Sepsis组明显提升(P<0.05,图2C、D),表明L-carnitine治疗可明显改善脓毒症小鼠心肌细胞的收缩功能。同时观察了L-carnitine对急性分离的心肌细胞钙瞬变的影响,结果发现,与Sham组比较,Sepsis组小鼠心肌细胞内钙离子浓度显著降低,钙瞬变幅度(△F/F0比值)下降了49.89%(P<0.05);与Sepsis组比较,L-carnitine组心肌细胞内钙离子浓度显著增加(P<0.05,图2E、F),△F/F0比值接近正常水平。以上结果表明,L-carnitine对脓毒症心肌收缩功能和钙瞬变有明显的改善作用。

2.3 L-carnitine改善脓毒症心肌线粒体脂肪酸氧化

L-carnitine在脂肪酸氧化中起重要作用,为此观察L-carnitine处理对脓毒症小鼠心肌组织脂肪酸氧化的作用。结果发现,与Sham组比较,Sepsis组小鼠心肌组织中TG和FFA的含量显著增加(P<0.05,图3A、B),而ATP的含量显著减少(P<0.05,图3C)。L-carnitine处理后可明显降低FFA和TG的水平,增加ATP的含量(P<0.05,图3)。通过LPS刺激HL-1心肌细胞12 h,在细胞水平复制脓毒症模型,结果与动物水平一致,



A: Sarcomere length changes in acutely isolated single cardiomyocytes under continuous electrical stimulation; B: Sarcomere contraction amplitude in acutely isolated single cardiomyocytes; C: Maximum sarcomere shortening velocity in acutely isolated single cardiomyocytes; D: Maximum sarcomere relaxation velocity in acutely isolated single cardiomyocytes; E: Intracellular calcium fluorescence intensity changes in acutely isolated single cardiomyocytes under continuous electrical stimulation; F: Calcium transient amplitude in acutely isolated single cardiomyocytes. a: P<0.05, compared with the Sham; b: P<0.05, compared with the Sepsis

图2 L-carnitine对脓毒症小鼠急性分离的心肌细胞收缩和钙瞬变的改善作用(n=8, $\bar{x}\pm s$)

Figure 2 Improvement of L-carnitine on contraction and calcium transients of acutely isolated cardiomyocytes from septic mice (n=8, $\bar{x}\pm s$)

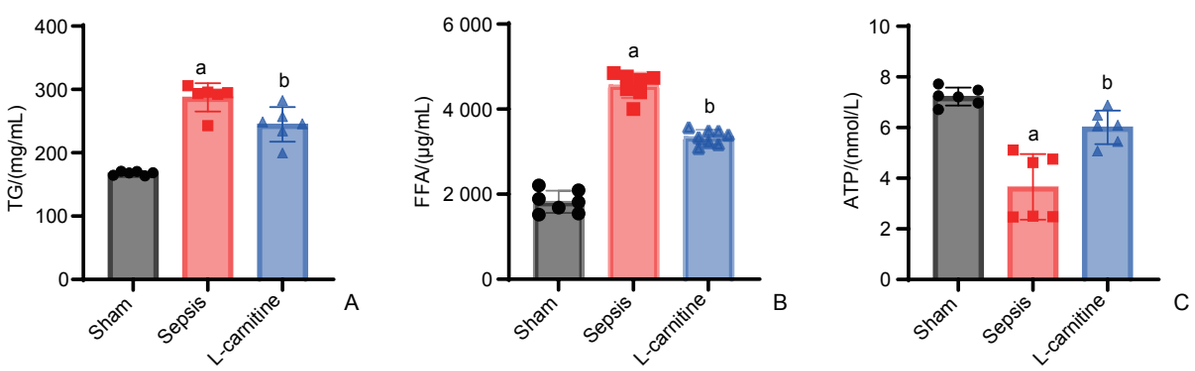
L-carnitine明显拮抗LPS诱导的FFA、TG增加和ATP含量降低(图4)。上述结果表明,L-carnitine能有效逆转脓毒症诱导的心肌细胞FFA转化和利用功能障碍,恢复其能量供应。

为明确L-carnitine对脓毒症心肌细胞脂肪酸氧化的影响,以棕榈酸-BSA(Palmitate-BSA)为底物,检测HL-1心肌细胞线粒体耗氧率(oxygen consumption rate, OCR)。正常对照(CTRL)组中,Palmitate-BSA组基础OCR显著高于BSA组(P<0.05,图5A、D),加入Eto后基础OCR显著降低(P<0.05,图5D),ΔOCR为(28.75±4.32) pmol/min(图5E),且CTRL组最大呼吸能力显著升高(P<0.05,图5F)、Eto处理后下降(P<0.05,图5F),提示正常心肌细胞可有效利用棕榈酸。

LPS处理组中,BSA与Palmitate-BSA组基础OCR无差异(图5B、D),Eto引起的ΔOCR仅(6.76±2.29) pmol/min(图5F);而LPS+L-carnitine组中,Palmitate-BSA组基础OCR显著高于BSA组(图5C、D),Eto处理后ΔOCR达(21.48±4.29) pmol/min(图5E),表明L-carnitine可恢复LPS损伤细胞对棕榈酸的氧化利用。此外,LPS组最大呼吸能力降低25.75%、ATP生成减少(P<0.05,图5F、G),L-carnitine可显著缓解(P<0.05,图5F)。综上,L-carnitine通过促进脂肪酸入线粒体,改善LPS所致心肌细胞脂肪酸氧化障碍。

2.4 L-carnitine通过CPT1A依赖性机制改善脓毒症心肌细胞脂肪酸氧化功能

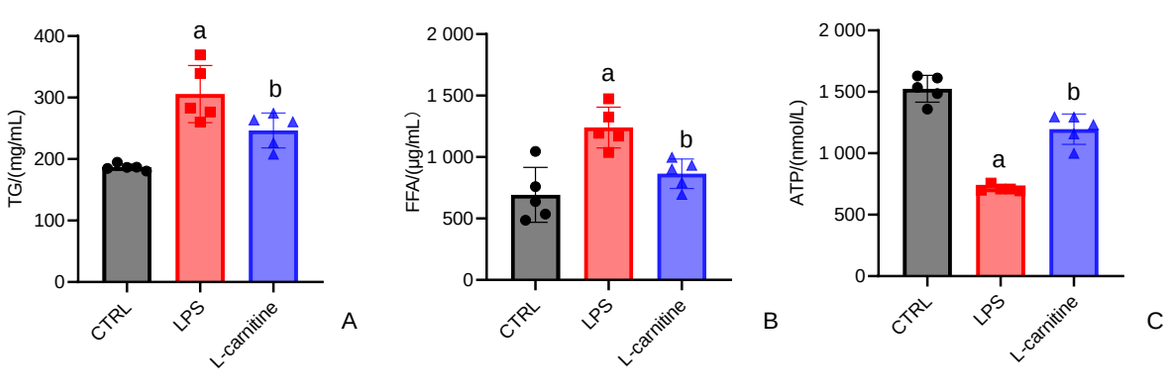
前期结果发现,CPT1A抑制剂Eto能逆转L-carnitine对LPS刺激心肌细胞的最大呼吸能力降低的改善作用,提示L-carnitine对心肌细胞FAO能力的改善作用与CPT1A有关。为探究L-carnitine改善脓毒症心肌细胞FAO的机制,检测了关键酶CPT1A的表达和活性。结果显示,LPS处理明显降低HL-1心肌细胞CPT1A的表达和活性,而L-carnitine处理能显著逆转这一效应(P<0.05,图6A、B)。使用BODIPY-FL-C16



A: Triglyceride (TG) content in myocardial tissue quantified by biochemical assay; B: Free fatty acid (FFA) content in myocardial tissue quantified by biochemical assay; C: Adenosine triphosphate (ATP) content in myocardial tissue quantified by biochemical assay. a: P<0.05, compared with the Sham; b: P<0.05, compared with the Sepsis

图3 L-carnitine对脓毒症小鼠心肌组织中TG、FFA和ATP水平的影响(n=8, $\bar{x}\pm s$)

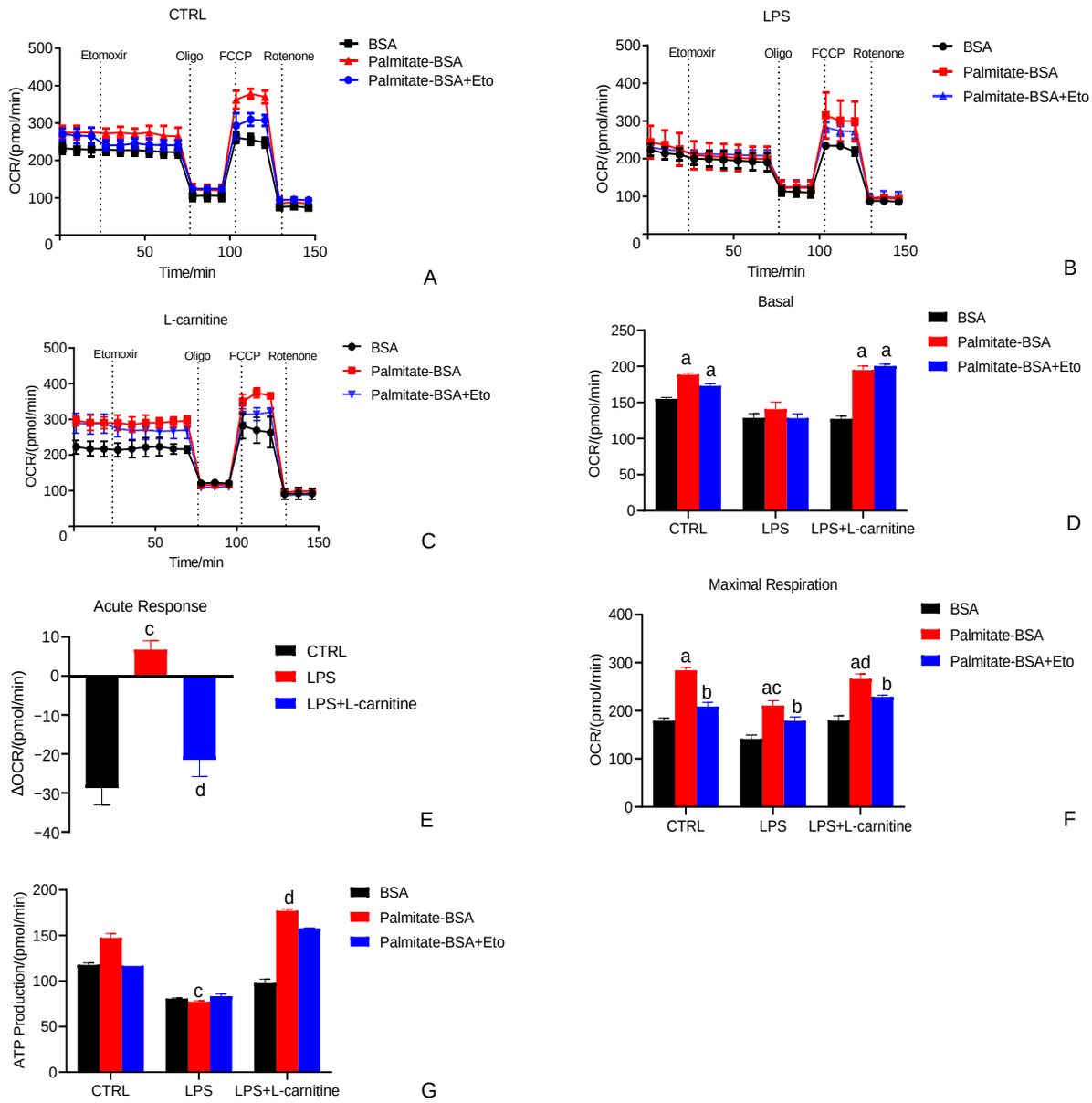
Figure 3 Effect of L-carnitine on TG, FFA, and ATP levels in myocardial tissue from septic mice (n=8, $\bar{x}\pm s$)



A: Triglyceride (TG) content in HL-1 cardiomyocytes quantified by biochemical assay; B: Free fatty acid (FFA) content in HL-1 cardiomyocytes quantified by biochemical assay; C: Adenosine triphosphate (ATP) content in HL-1 cardiomyocytes quantified by biochemical assay. a: P<0.05, compared with the CTRL; b: P<0.05, compared with the LPS

图4 L-carnitine对LPS处理的HL-1心肌细胞中TG、FFA和ATP水平的影响

Figure 4 Effect of L-carnitine on TG, FFA, and ATP levels in HL-1 cardiomyocytes treated with LPS

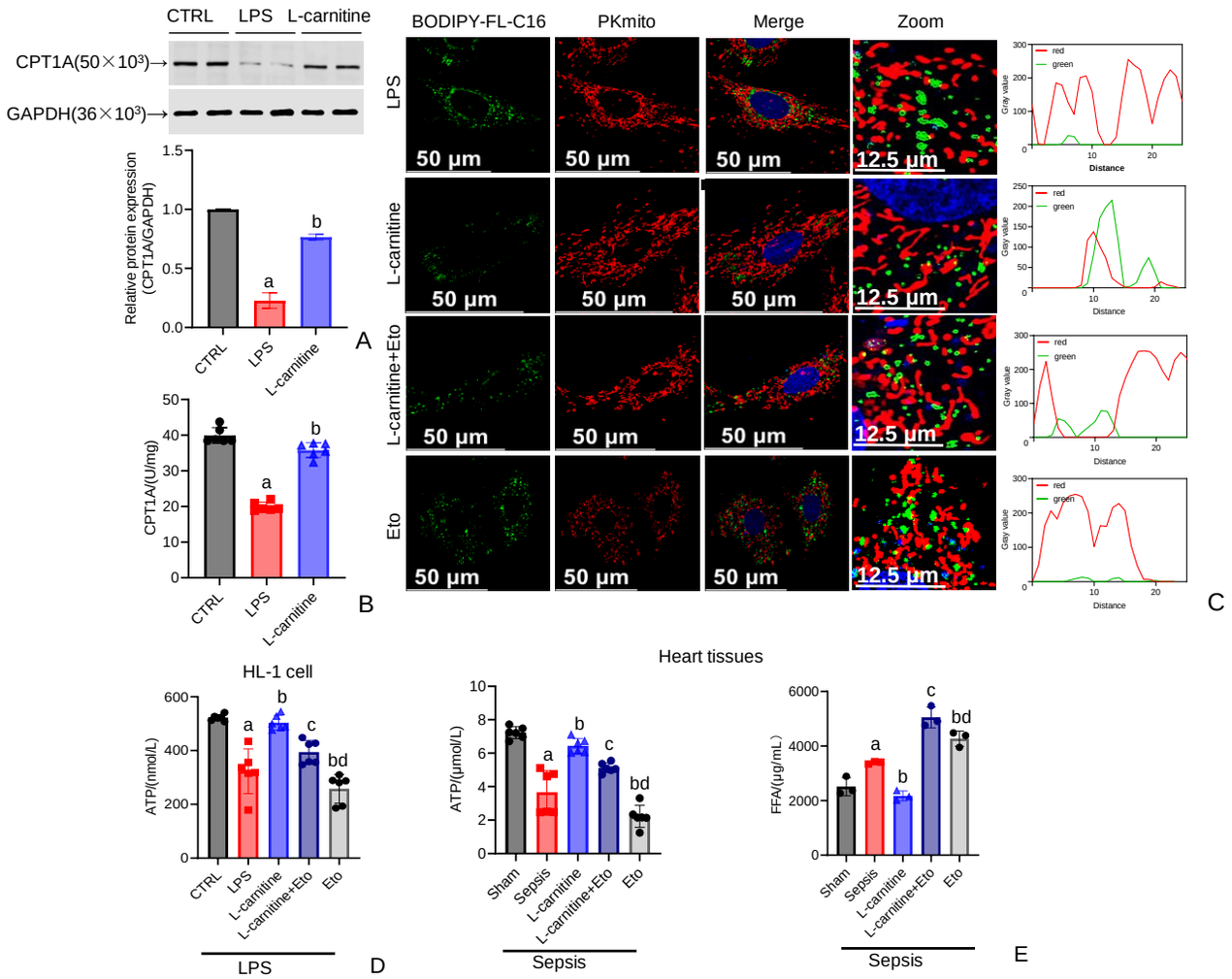


A~C: Dynamic curves of oxygen consumption rate (OCR) in HL-1 cardiomyocytes from CTRL (A), LPS (B), and L-carnitine (C) groups by cell energy metabolism analysis system; D: Basal oxygen consumption rate in HL-1 cardiomyocytes by cell energy metabolism analysis system; E: Acute OCR response in HL-1 cardiomyocytes by cell energy metabolism analysis system; F: Maximal respiratory capacity in HL-1 cardiomyocytes by cell energy metabolism analysis system; G: ATP production in HL-1 cardiomyocytes by cell energy metabolism analysis system. a: $P<0.05$, compared with the BSA; b: $P<0.05$, compared with the Palmitate-BSA; c: $P<0.05$, compared with the CTRL; d: $P<0.05$, compared with the LPS

图5 L-carnitine对LPS处理的HL-1心肌细胞长链脂肪酸氧化能力的影响

Figure 5 Effect of L-carnitine on long-chain fatty acid oxidation capacity of HL-1 cardiomyocytes treated with LPS

标记棕榈酸,观察其在HL-1心肌细胞的分布。结果发现,LPS组绿色荧光标记的棕榈酸主要在细胞质异常聚集,与线粒体共定位减少(图6C);L-carnitine细胞质棕榈酸减少,与线粒体共定位增加,该效应可被Etomoxir阻断(图6C)。同时,与L-carnitine组相比,L-carnitine+Eto组HL-1心肌细胞中ATP的含量也显著降低($P<0.05$,图6D)。体内实验结果也显示,与L-carnitine组相比,L-carnitine+Eto组小鼠心肌组织中ATP水平降低($P<0.05$,图6E),FFA水平升高($P<0.05$,图6E)。这些结果表明L-carnitine通过CPT1A依赖性机制促进脂肪酸进入线粒体进行β-氧化,从而改善脓毒症心肌细胞FAO功能障碍。



A: CPT1A protein expression in HL-1 cardiomyocytes detected by Western blotting; B: CPT1A activity in HL-1 cardiomyocytes determined by biochemical assay; C: Subcellular distribution of fatty acids in the cytoplasm and mitochondria of HL-1 cardiomyocytes analyzed by immunofluorescence and biochemical fractionation; D: Changes in ATP content in HL-1 cardiomyocytes quantified by biochemical assay; E: Changes in ATP and FFA contents in septic mouse myocardial tissue measured by biochemical assay. a: $P<0.05$, compared with the CTRL or Sham; b: $P<0.05$, compared with the LPS or Sepsis; c: $P<0.05$, compared with the L-carnitine; d: $P<0.05$, compared with the L-carnitine+Eto

图6 L-carnitine通过CPT1A依赖性机制改善脓毒症心肌细胞脂肪酸氧化功能

Figure 6 L-carnitine improves fatty acid oxidation function in LPS-treated HL-1 cardiomyocytes and septic mouse myocardial tissue via a CPT1A-dependent mechanism

3 讨论

3.1 左旋肉碱对脓毒症心肌收缩功能的改善作用

脓毒症诱导的心脏功能障碍是脓毒症最严重的并发症,病死率高达70%~90%[14]。心肌收缩功能障碍是脓毒症致心脏功能障碍的重要特征,其发生机制涉及炎症反应、氧化应激、钙调节紊乱、自主神经系统失调、线粒体功能障碍等[15]。针对上述机制,已有研究发现部分药物对脓毒症心肌收缩功能有一定改善作用,如正性肌力药多巴酚丁胺、线粒体保护剂、炎性因子抑制剂等,但临床治疗效果均不理想[16]。因此,深入研究脓毒症致心肌收缩功能障碍的发生机制并开发新型治疗药物具有重要临床意义。

左旋肉碱作为一种重要的营养物质和代谢调节剂,在改善心肌能量代谢、减轻氧化应激和炎症反应等方面具有潜在的治疗价值。研究表明,左旋肉碱可减轻大鼠心肌缺血再灌注损伤,降低氧化应激标志物(如MDA)和凋亡标志物(如Caspase-3)[16]。在肺动脉高压中,补充左旋肉碱可以改善心输出量,增加右心室射血分数并降低血管阻力[17]。本研究利用盲肠结扎穿孔术建立脓毒症小鼠模型,发现左旋肉碱治疗可以明显恢复脓毒症心肌细胞收缩力,降低心肌损伤标

志物的水平,改善心肌功能,恢复血压,从而改善脓毒症小鼠结局。然而,脓毒症期循环系统的稳定性,如休克的程度及持续的时间对心脏功能亦有显著影响,左旋肉碱是否对不同程度脓毒症休克诱导的心肌功能障碍依然有效,仍需进一步研究。

3.2 左旋肉碱对脓毒症心肌能量代谢的调控作用

心肌能量供应和收缩相关蛋白是决定心肌细胞收缩功能的两个重要环节。正常情况下,脂肪酸β氧化是心肌细胞产生能量的主要方式,占心肌能量来源的60%~70%[18]。脓毒症后,心肌细胞脂肪酸氧化严重受损,不完全的脂肪酸氧化可能会产生一些中间产物的堆积,进一步加重心肌细胞的代谢负担,进而影响心肌收缩功能[19]。本研究也发现,脓毒症小鼠心肌组织和心肌细胞中ATP含量减少,而TG和FFA水平增加,线粒体脂肪酸氧化明显受损,导致细胞内脂质过度积累,进一步影响心肌细胞收缩功能,而左旋肉碱处理可逆转这些变化,改善脂肪酸氧化,恢复心肌组织的能量供应,进而改善心肌收缩功能。而左旋肉碱是否可以通过直接作用于收缩相关蛋白从而调节心肌细胞收缩力,仍需进一步研究。

3.3 左旋肉碱对脓毒症心肌钙瞬变的改善及潜在机制

此外,本研究还发现左旋肉碱可以改善脓毒症心肌细胞钙瞬变。钙瞬变是心肌细胞兴奋-收缩耦联的关键环节,依赖于肌浆网Ca^{2+}-ATP酶(sarco endoplasmic reticulum Ca^{2+}-ATPase,SERCA)的活性,而该活性需要ATP供能[20]。脂肪酸氧化是心肌细胞产生ATP最多的途径,因此,本研究推测左旋肉碱可能通过促进脓毒症心肌细胞脂肪酸氧化增加ATP合成,从而增强SERCA功能,改善钙循环。另一方面,脂肪酸氧化产生的乙酰辅酶A可能通过组蛋白乙酰化调控SERCA2a、受磷蛋白PLN等钙处理相关基因的表达[21]。左旋肉碱是否通过直接影响这些钙调节相关蛋白的表达或活性从而影响心肌细胞钙瞬变,仍需进一步研究。

3.4 CPT1A在左旋肉碱调控脓毒症心肌收缩机制中的核心作用

基础研究表明,CPT1A是长链脂肪酸转运的限速酶,也是左旋肉碱发挥作用的重要靶点[22]。本研究发现脓毒症后CPT1A的表达显著下调,左旋肉碱治疗能有效恢复其表达水平,而CPT1A抑制剂Eto的应用削弱了左旋肉碱对脂肪酸进入线粒体的促进能力,逆转了脓毒症诱导的心肌细胞脂肪酸氧化功能障碍的改善作用,表明左旋肉碱通过CPT1A依赖性机制促进脂肪酸进入线粒体进行β-氧化,从而改善脓毒症心肌细胞脂肪酸氧化功能障碍。因此,双重靶向左旋肉碱-CPT1A轴可能进一步改善脓毒症心肌收缩功能障碍。

本研究从动物和细胞层面揭示了左旋肉碱改善脓毒症心肌收缩功能障碍的作用机制,为临床治疗提供了新的潜在策略。现有临床研究显示,左旋肉碱(左卡尼汀)在急性心肌梗死及冠脉搭桥术后等能量代谢障碍患者中已展现出治疗潜力[23-24]。一项针对脓毒症危重患者的随机对照试验也表明,左旋肉碱可缓解炎症、增强抗氧化能力并降低死亡率[25],提示其在脓毒症心肌病治疗中具有一定前景,但仍需更多临床验证。此外,本研究仍存在一定的局限性:①体外实验采用LPS刺激HL-1细胞建立脓毒症心肌损伤体外模型,该细胞系与原代心肌细胞存在一定的代谢差异,且LPS处理仅部分模拟了脓毒症病理过程,后续需采用原代心肌细胞或用脓毒症血清刺激进一步验证。②虽使用CPT1A抑制剂Eto表明左旋肉碱作用依赖于CPT1A,但该抑制剂可能存在脱靶效应,独立于CPT1A调控细胞代谢,未来应通过CPT1A条件性敲除模型进一步明确其机制。

综上所述,左旋肉碱可通过CPT1A依赖性机制促进脂肪酸进入线粒体进行β-氧化,从而改善脓毒症小鼠心肌细胞的脂肪酸氧化功能,从而改善心肌收缩功能。

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