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The down-regulation of cardiac contractile proteins underlies myocardial depression during sepsis and is mitigated by carbon monoxide

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ABSTRACT

The aim of this study is to investigate the mechanism underling cardiac dysfunction during sepsis, as well as the possible amelioration of this dysfunction by exogenous carbon monoxide (CO) administration. For this purpose, rats (six-week-old, male, Sprague-Dawley) were administered LPS (15 mg/kg body weight, i.p. 6 h) and/or CORM (30 mg/kg, i.p.). The decreased left ventricular ejection fraction (EF) observed in LPS group rats was recovered in the LSP + CORM group, confirming the protective role of CO against sepsis-induced myocardial depression. Proteomic as well as immunoblot analysis showed that the levels of myosin heavy and light chains (MHC and MLC) as well as α-cardiac actin (ACTC) were decreased in the LPS group, and these decreases were mitigated in the LSP + CORM group, suggesting that the amounts of major contractile proteins are decreased in depressed myocardium. Not only LPS-induced inflammatory cytokine (TNFα and IL-1β) production but also the decrease in myofilament proteins was mitigated by CORM. These results confirm the protective action of exogenously administered CO against myocardial depression during sepsis, and reveal a novel mechanism underling cardiac dysfunction during sepsis.

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1. Introduction

Sepsis is the most prominent cause of death in hospitals, especially in the ICU due to the lack of effective therapeutics [1,2]. Sepsis is characterized by systemic inflammatory response syndrome (SIRS), which leads to septic shock and multiple organ failure (MOF) [1]. During septic shock, microcirculatory dysfunction is often followed by myocardial depression caused by damage to cardiomyocytes by excessive inflammatory cytokines as well as aberrant hemodynamics [3–5]. In addition, mitochondrial damage has attracted much attention as an intrinsic mechanism for myocardial depression [5]. Mitochondrial damage is observed widely in various organs/tissues in humans and in experimental animal models of sepsis [6–8], and should be linked directly to contractile failure due to the massive demand for mitochondrial energy production for cardiomyocyte contraction.

In addition to cellular sources of energy such as ATP and creatine phosphate, cardiomyocyte elasticity is essential for the contraction of the heart. Striated muscle sarcomeres, which are composed of thin (actin) and thick (myosin) filaments, are responsible for contraction. In cardiomyocytes, the thin actin filaments are polymers of the α-cardiac actin (ACTC) monomer, and the thick myosin filaments are composed of a myosin complex containing myosin heavy and light chains (MHC and MLC) [9,10]. MLC1/3 serves as the non-phosphorylatable constitutive MLC while MLC2 is activated by MLCK (MLC kinase)-dependent phosphorylation in response to inotropic stimulation to enhance cardiac output [11]. The abundance, composition, and activity of cardiac contractile proteins are tightly regulated to maintain proper performance of the heart [12,13]. Upon cardiac stress, damaged sarcomeric proteins are eliminated through a panel of protein degradation systems including the ubiquitin-proteasome system [14]. Muscle RING-finger protein-1 (MuRF1) and atrogin-1 (also called as MAFbx) are E3 ubiquitin ligases responsible for the degradation of sarcomeric proteins [15–18].

Many laboratories, including our own, have reported the emerging roles of carbon monoxide (CO) in protecting liver [19], lung [20], and heart [21] against sepsis. Protection of these major organs, as well as individual organisms, against sepsis by the inhalation of appropriate doses of CO has recently emerged as a

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promising therapeutic against sepsis [22]. The advantageous effects of CO can be attributed mainly to the potent anti-inflammatory effects of CO: CO has been shown to suppress the LPS-induced production of proinflammatory cytokines (TNFα and IL-1β), while boosting anti-inflammatory cytokine (IL-10) production [23].

Here we report that the major sarcomeric myofilament proteins, cardiac myosin and actin, are down-regulated both in their protein and mRNA levels in the heart of rats administered LPS. The co-administration of CO-releasing molecule (CORM) [24] not only suppresses tissue inflammation but also mitigates the down-regulation of cardiac myosin as well as actin. These results suggest that the decreased abundance of contractile proteins should underlie myocardial depression during sepsis.

2. Materials and methods

2.1. Animals

All protocols were approved by the Institutional Animal Care and Use Committee of the Tokyo Medical and Dental University. Six-week-old rats (Sprague-Dawley, male, obtained from Charles River Laboratories International, Inc.) were injected intraperitoneally (i.p.) with 15 mg/kg body weight LPS (L-2630, Sigma-Aldrich, St. Louis, MO), and the hearts were excised 6 h later. In some experimental groups, CORM [CORM-3, $Ru(CO)_3Cl$(Glycinate), Sigma-Aldrich] was administered at a dose of 30 mg/kg body weight 1 h prior to the administration of LPS [24]. Each experimental group consisted of 3–4 independent animals.

2.2. Cell culture

H9c2 rat cardiomyoblasoma cells were grown in DMEM supplemented with FBS (10%), streptomycin (100 μg/ml), and penicillin (100 U/ml) in 5% CO_2 atmosphere in a humid incubator. LPS and CORM were added to the medium at final concentrations of 5 μg/ml and 100 μM, respectively.

2.3. Histochemistry

PFA-fixed and paraffin-embedded heart tissues were deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E).

2.4. Echocardiography

Cardiac function was determined as previously described [21]. In brief, transthoracic M-mode images of the left ventricle (LV) were acquired using echocardiography (Noblus, Hitachi-Aloka Medical, Japan). The ejection fraction (EF) and fractional shortening (FS) were calculated from the digital images. These echocardiographic measurements were performed three times for each rat 6 h after LPS injection (n = 4).

2.5. Identification of protein by MALDI-TOF-MS

Equal amounts of tissue lysate were subjected to Mini-PROTEAN TGX Precast Protein Gels (4–20%, Bio-Rad), followed by CBB staining. Several bands were excised with a knife, de-stained, and dried with acetonitrile. Dehydrated gel pieces were rehydrated and reduced with 10 mM DTT, S-alkylated with 55 mM iodoacetamide, and digested with 10 ng/ml trypsin (sequence grade, modified, Promega, Madison, WI) at 37 °C overnight. The resultant trypsin-digested peptides were subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis. α-Cyano-4-hydroxycinnamic acid (Bruker) was used as the matrix and MALDI-TOF-MS analysis was performed using an ultrafleXtreme (Bruker). The peak list files obtained were subjected to the Mascot Server search engine (Matrix science, Boston, MA) against SwissProt database.

2.6. Immunoblot analysis

Hearts were excised from sacrificed rats and homogenized in STE buffer [0.32 M sucrose, 10 mM Tris-HCl, 5 mM EDTA, 50 mM NaF, 2 mM Na_3VO_4, and protease inhibitors (Complete, Roche Diagnosis, Indianapolis, IN)]. Equal amounts of lysates were electrophoresed in SDS gels, followed by blotting to PVDF membranes (Merck Millipore, Billerica, MA). The resultant blots were incubated with 1st antibodies (overnight at 4 °C), followed by incubation with appropriate 2nd antibodies (1 h at room temperature). The following 1st and 2nd antibodies were used: anti-MHC (sc-20641, Santa Cruz Biotechnologies, Dallas, TX), anti-MLC (sc-365243, Santa Cruz Biotechnologies), anti-α-cardiac actin (GTX101876, GeneTex, Irvine, CA), anti-GAPDH (MAB374, Merck Millipore), anti-atrogin-1 (AP2041, ECM biosciences, Versailles, KY), anti-MURF1 (#4305, Cell Signaling Technology, Berverly, MA), anti-caspase-1 (ab179515, abcam, Cambridge, MA), and HRP-conjugated anti-IgG antibodies (Promega). The antigens were visualized with a Western Lightning Chemiluminescence Reagent Plus Kit (PerkinElmer Life Sciences, Boston, MA), and signal intensities were calculated using an image analyzer (CS Analyzer, ATTO, Tokyo, Japan).

2.7. qPCR

Quantitative reverse transcriptase-mediated PCR (qPCR) was performed as follows. First, total RNA was extracted from the tissue using TRIzol reagent (Invitrogen, Carlsbad, CA). Then, cDNA was synthesized with oligo$(dT)_{15}$ as a primer using SuperScript II reverse transcriptase (Invitrogen). A StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA) was used for quantitative analysis. SYBR green (GoTaq qPCR master mixture, Promega) was used as the fluorescence dye. The PCR conditions were as follows: 95 °C for 20 s followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s. Relative levels of transcripts were calculated by the 2-ΔΔCτ method. The primers used were: 5′-CATCCGTTCTCTACCCAGCC-3′ and 5′-AATTCTGAGCCCGGAGTTGG-3′ for TNFα; 5′-GCAGCTTTC-GACAGTGAGGA-3′ and 5′-TCATCTGGACAGCCCAAGTC-3′ for IL-1β; 5′-AATAAAAGCAAGGCAGTGGAGC-3′ and 5′-ATTCATGGCCTTGTA-GACACCT-3′ for IL-10; 5′-GTCAGCAGAACAGTAAAATAGAGGA-3′ and 5′-GGCTTCCTCTAGCCTCTCAC-3′ for αMHC; 5′-CAGAGCA-GATCGCCCTCAAG-3′ and 5′-CTGCAGTCGCAGTAGGTTCT-3′ for βMHC; 5′-AGGAGACCATTCTCAACGCC-3′ and 5′-GGGGAAAAGCTGCGAACATC-3′ for MLC2; 5′-TGGCACCAAAGAAA-GACGTG-3'and 5′-CCGGTTCTGTCGAAGAGGAG-3'for MLC1/3; 5′-CTTTGGTGTGCGACAATGGC-3'and 5′-GCTCTGAGCCTCGTCACCTA-3′ for α-cardiac actin (ACTC); 5′-AGCTTGTGCGATGTTACCCA-3'and 5′-GGTGAAAGTGAGACGGAGCA-3′ for atrogin-1; 5′-CCCTGATCCTC-CAGTACCGA-3′ and 5′-ATGCTCTTGATGAGCGGCTT-3′ for MuRF1. 5′-GGCTCTCTGCTCCTCCCTGTTCTA-3′ and 5′-TGCCGTTGAACTTGCCGTGGG-3′ for GAPDH.

2.8. Statistical analysis

Data are expressed as means and S.E. (n = 3–4). Multiple group comparisons were made by analysis of variance (ANOVA), followed by Tukey-Kramer for post hoc comparisons. Statistical differences were considered significant at $p < 0.05$.

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