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Cardiac-specific overexpression of thioredoxin 1 attenuates mitochondrial and myocardial dysfunction in septic mice

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ABSTRACT

Sepsis-induced myocardial dysfunction is associated with increased oxidative stress and mitochondrial dysfunction. Current evidence suggests a protective role of thioredoxin-1 (Trx1) in the pathogenesis of cardiovascular diseases. However, it is unknown yet a putative role of Trx1 in sepsis-induced myocardial dysfunction, in which oxidative stress is an underlying cause. Transgenic male mice with Trx1 cardiac-specific overexpression (Trx1-Tg) and its wild-type control (wt) were subjected to cecal ligation and puncture or sham surgery. After 6, 18, and 24 h, cardiac contractility, antioxidant enzymes, protein oxidation, and mitochondrial function were evaluated. Trx1 overexpression improved the average life expectancy (Trx1-Tg: 36, wt: 28 h; $p = 0.0204$). Sepsis induced a decrease in left ventricular developed pressure in both groups, while the contractile reserve, estimated as the response to β-adrenergic stimulus, was higher in Trx1-Tg in relation to wt, after 6 h of the procedure. Trx1 overexpression attenuated complex I inhibition, protein carbonylation, and loss of membrane potential, and preserved Mn superoxide dismutase activity at 24 h. Ultrastructural alterations in mitochondrial cristae were accompanied by reduced optic atrophy 1 (OPA1) fusion protein, and activation of dynamin-related protein 1 (Drp1) (fission protein) in wt mice at 24 h, suggesting mitochondrial fusion/fission imbalance. PGC-1α gene expression showed a 2.5-fold increase in Trx1-Tg at 24 h, suggesting mitochondrial biogenesis induction. Autophagy, demonstrated by electron microscopy and increased LC3-II/LC3-I ratio, was observed earlier in Trx1-Tg. In conclusion, Trx1 overexpression extends antioxidant protection, attenuates mitochondrial damage, and activates mitochondrial turnover (mitophagy and biogenesis), preserves contractile reserve and prolongs survival during sepsis.

Abbreviations: ANOVA, analysis of variance; ATP, adenosine triphosphate; CK-MB, creatine kinase MB; Drp1, dynamin-related protein 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LC3, microtubule associated protein 1A/1 B light chain 3; Mfn1, 2 mitofusin 1 2; MOMP, mitochondrial outer membrane permeability; NADPH, nicotinamide adenine nucleotide phosphate; NF-κB, nuclear factor-κB; Nrf, nuclear respiratory factor; OPA 1, optic atrophy protein 1; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PGC-1, peroxisome proliferator-activated receptor-α-coactivator-1; ROS, reactive oxygen species; SOD, superoxide dismutase; TFAM, mitocondrial transcription factor A; Trx, thioredoxin; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; UCP, uncoupling protein; VDAC, voltaje-dependent anion channel.

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1. Introduction

Mortality related to sepsis mainly results from the dysfunction and failure of vital organs, including the heart. Current evidence indicates that there are many mechanisms involved in myocardial dysfunction, including inflammatory response, alterations in intracellular calcium homeostasis and adrenergic signaling, mitochondrial dysfunction, and apoptosis (Flierl et al., 2008; Flynn et al., 2007; Rudiger and Singer, 1992). Oxidative stress seems to play a major role among the pathophysiological mechanisms of sepsis, and in the development and progression of sepsis-induced organ failure. There is plenty of evidence supporting a positive correlation between oxidative damage and organ injury (Gonzalez et al., 2014; Ritter et al., 2003; Wheeler, 2011). Moreover, many studies demonstrate outcome improvement in sepsis after antioxidant supplementation and reinforcement of endogenous antioxidant defenses (Baumgart et al., 2009; Ceylan-Isik et al., 2010; Lowes and Galley, 2011).

In experimental models of sepsis, the impact of antioxidant therapy has been extensively evaluated, but little specific information on cardiac dysfunction in sepsis is available, or it is inconclusive. The myocardium exhibits two thiol-based major antioxidant defenses to maintain a reduced intracellular redox state, such as thioredoxin (Trx) and glutaredoxin (Grx) systems (Berndt et al., 2007). The Trx system consists of Trx, NADPH, and Trx reductase (TrxR). Trx is a small multifunctional redox-active protein involved in cellular redox homeostasis and cell survival (Holmgren and Lu, 2010). There are three isoforms of Trx depending on their localization, such as Trx1 with its cytosolic and nuclear forms, Trx2 mitochondrial form, and Sp-trx, which is expressed in testes (Miranda-Vizuete et al., 2001). Trx1 is the most intensively studied, because it has many biological functions. It reduces peroxiredoxin or oxidized proteins, interacts with transcription factors (Powis and Montfort, 2001), participates in control of apoptosis through binding to apoptosis signal regulating kinase-1 (ASK-1), and modulates other redox-regulated proteins like caspases through the control of protein S-nytrosilation and denitrosylation (Li et al., 2013).

Several responses allow the mitochondrial network to adapt to stress and loss of membrane potential. These include mitochondrial fission and fusion, mitophagy, and mitochondrial biogenesis (Youle and van der Bliek, 2012). Mitochondrial dynamics is a recent topic in cardiac physiology (Song and Dorn, 2015; Ikeda et al., 2015; Cimolai et al., 2015). The proteins involved in mitochondrial fusion (mitofusins [Mfn 1 and 2] and optic atrophy protein 1 [OPA1]) and fission (dynamin related protein 1 [Drp1]) are highly expressed in cardiomyocytes, and are required for mitochondrial biogenesis and the quality control of the organelles. In particular, mitochondrial fission allows for selective segregation of damaged mitochondria, which are afterwards eliminated by autophagy (or mitophagy).

Some molecular research studies on cardiac-specific overexpression of Trx1 have demonstrated that proteins associated to mitochondrial permeability transition pore (MPTP), and contractile apparatus are selective targets for facilitating muscle contraction (Fu et al., 2009), and Trx1 even upregulates mitochondrial proteins and enhances mitochondrial function, possibly by activating genes related with mitochondrial biogenesis (Ago et al., 2006).

Additional evidence has emphasized the importance of Trx1 in cardiovascular diseases, including heart ischemia-reperfusion injury, contractile dysfunction, atherosclerosis and heart failure (Ahsan et al., 2009; Mahmood et al., 2013; Nicholson et al., 2013).

The aim of this study was to evaluate the protective role of Trx1 in CLP-induced myocardial dysfunction, focusing on cardiac contractility, mitochondrial function, dynamics and biogenesis.

2. Materials and methods

2.1. Cardiac-specific thioredoxin1-overexpressing mice

Transgenic male mice with Trx1 cardiac-specific overexpression (Trx1-Tg) (22–32 g) were used. The mice were donated for research purposes to the Institute of Cardiovascular Physiopathology, Department of Pathology, School of Medicine, Universidad de Buenos Aires, Argentina (courtesy of Sadoshima J., New Jersey Medical School, Rutgers University, Newark, USA). As previously described (Yamamoto et al., 2003), Trx1-Tg mice were generated on an FVB background, using the α-myosin heavy chain promoter to achieve cardiac-specific expression.

2.2. Cecal ligation and puncture (CLP) model

The model of cecal ligation and puncture (CLP) in rodents has been extensively used to research the clinical settings of sepsis and septic shock. This model produces a hyperdynamic, hypermetabolic state that can lead to a hypodynamic, hypometabolic stage, and eventual death (Hubbard et al., 2005). It is based on the disruption of the intestinal barrier by means of surgical procedures. Animal experiments were performed in accordance with the Principles of Laboratory Animal Care. The Institutional Animal Care and Research Committee of the Universidad de Buenos Aires approved all animal procedures. We made every possible effort to minimize animal suffering and to reduce the number of animals used. Animals were given access to food and water *ad libitum*. All the animals were fasted for 16 h before any surgical procedure. The mice were anesthetized with an intraperitoneal ketamine ($100\ mg\ kg^{-1}$) and xylazine ($5\ mg\ kg^{-1}$) mixture. Under aseptic conditions, a 1.5–2.0 cm midline incision was performed to allow exposure of the cecum. The cecum was tightly ligated, and perforated twice with a 21-gauge needle. The cecum was then gently squeezed to extrude a small amount of fecal content from the perforation sites to the abdominal cavity. A sham surgery (laparotomy and cecum exposure) was performed as control. The animals were resuscitated with normal saline (NaCl 0.9%, 1 ml subcutaneous) and pain medication (tramadol 20 μg/g BW) was administered immediately after CLP. The animals were sacrificed 6, 18, or 24 h after CLP or the sham operation and the hearts were isolated for posterior analyses, in order to evaluate the hyperdynamic (6 h) and hypodynamic (18–24 h) stages of sepsis.

2.3. Survival experiments

Survival rate was tested in seven animals per group, which underwent the CLP procedure described above. Mortality was monitored over a period of 72 h.

2.4. Biochemical parameters

Blood samples were taken from mammary arteries without anticoagulants. Serum aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) activity were determined by the rate of decrease in NADH measured at 340 nm. Creatine kinase, CK, and CK-MB were assessed by CK-NAC UV method. Lactate dehydrogenase activity, LDH, was evaluated by direct reduction of NADH. All previous measurements were carried out by bioanalytical standard Wiener lab diagnostic kits. Serum biochemical parameters were used as markers of sepsis-related organ damage and evolution.

2.5. Myocardial contractility

After 6, 18, or 24 h of the surgically induced peritonitis or sham procedures, the mice were anesthetized by an intraperitoneal injec-

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