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The protective role of neuregulin-1: A potential therapy for sepsis-induced cardiomyopathy

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ABSTRACT

The extremely high mortality of sepsis in intensive care units, caused primarily by sepsis-induced cardiomyopathy, is a pressing issue. Current studies have revealed the importance of the neuregulin-1 (NRG-1)/ErbB signaling axis at the cardiovascular level and the positive effect of NRG-1 on cardiac function in patients with heart failure. To investigate the protective mechanism of NRG-1 against myocardial injury in septic rats, a cecal ligation and puncture (CLP) model was applied. Animals were administered either a vehicle or recombinant human NRG-1 (rhNRG-1, 10 μg/kg). Their survival rates were noted 24 h after CLP. The hemodynamic method was used to evaluate their cardiac function. The myocardial morphology was observed. An enzyme-linked immunosorbent assay was used to detect the level of cardiac troponin-T (cTn-T), cytokines, and angiotensin II (Ang II) in the serum and myocardium. Compared with the vehicle, rhNRG-1 improved survival of rats and prevented hemodynamic derangement, as reflected in the increased mean arterial pressure, left ventricular systolic pressure, ± dp/dt max, and decreased left ventricular end-diastolic pressure ($P < 0.05$). Furthermore, the serum levels of cTn-T and pro-inflammatory cytokines (tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6) were significantly increased in vehicle-treated rats but reduced in rhNRG-1-treated rats. The latter also showed decreased concentration of macrophage inhibitory factor and Ang II in the myocardium ($P < 0.05$). These results suggest that NRG-1 improved cardiac function and protected cardiomyocytes of rats from CLP-induced sepsis by suppressing the immune inflammatory response and excessive activation of the renin–angiotensin–aldosterone system. Ultimately, NRG-1 increased the survival rate of rats.

1. Introduction

According to The Third International Consensus Definitions Task Force, sepsis is defined as a "life-threatening organ dysfunction due to a dysregulated host response to infection" (Seymour et al., 2016). Heart is one of the most frequently affected organs. Approximately 50% of patients diagnosed with sepsis presented with signs of myocardial dysfunction (Charpentier et al., 2004), with a higher mortality ranging from 70% to 90% (Vieillard-Baron et al., 2008).

Given the technical and methodological limitations, sepsis-induced cardiomyopathy currently encompasses left ventricular dilatation, that is, depressed ejection fraction as a "reversible" condition. The underlying mechanism of cardiomyopathy during sepsis has not been fully elucidated yet. However, the main proposed mechanisms include myocardial injury, circulation of cardio-depressants, autonomic dysregulation, endothelial dysfunction and coronary microcirculatory changes, mitochondrial dysfunction and apoptosis, disorders of intracellular calcium regulation, nitric oxide-mediated depression, release of matrix metalloproteinases, and the effect of Toll-like receptors (Zaky et al., 2014). Novel therapeutic drugs for treating sepsis-induced cardiomyopathy specifically target the suppression of the inflammatory response via cytokines, such as tumor necrosis factor-α and interleukin-1 beta (IL-1β) (Abraham et al., 2001; Opal et al., 1997). However, therapeutic strategies that seemed promising in animal studies have largely been ineffective in human clinical trials. Existing treatments are not effective possibly due to the multifactorial and multiple mechanisms of sepsis-induced cardiomyopathy. Thus, it may be difficult to target a single factor to treat the whole disease. Therefore, effective, multi-target drugs are needed to improve clinical outcomes.

Neuregulin-1 (NRG-1) belongs to the family of epidermal growth factors. It is involved in cell survival, proliferation,

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migration, and differentiation via the activation of tyrosine kinase receptors of the ErbB family (ErbB2, ErbB3, and ErbB4) (Rupert and Coulombe, 2015). Recently, the therapeutic potential of neuregulin in cardiovascular disease has been proposed. Studies have shown that NRG-1 protects the myocardium and restores cardiac function via several pathways. At the cellular level, NRG-1 protected rats from doxorubicin (DOX)-induced myocardial cell toxicity (Liu et al., 2014). In several models of acute heart failure, NRG-1 exerted a cardioprotective effect, which included improving the overall cardiac function (Formiga et al., 2014) and reducing the myocardial infarct size (Fang et al., 2010) and left ventricle remodeling (Guo et al., 2012) by increasing cardiomyocyte proliferation and microvasculature of the infarcted myocardium, and alleviating mitochondrial dysfunction and myocyte apoptosis. Moreover, NRG-1 therapy showed beneficial effects on cardiac function and survival in various in vivo models of chronic heart failure (Liu et al., 2006). In addition, injection of recombinant NRG-1β into the rostral ventrolateral medulla in rats was found to lower blood pressure, heart rate, and renal sympathetic nerve activity (Matsukawa et al., 2011). Thus, NRG-1/ErbB signaling in the central nervous system plays a key role in cardiovascular homeostasis.

Although extensively studied, NRG-1/ErbB signaling in the heart is yet to be explored as a potential treatment of cardiomyopathy in sepsis. As the protective mechanism of NRG-1 shares certain common features with sepsis-induced cardiomyopathy, we speculate that NRG-1 may play a cardioprotective role during sepsis.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats weighing 250–300 g (Hunan SJA Laboratory Animal Co., Ltd., China) were housed in standard plastic cages with sawdust bedding placed in an air-conditioned room at 22 ± 1 °C. Standard rat food and tap water were provided ad libitum. The animal experiments and procedures were approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University. They were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1996).

2.2. Experimental design

The rats were randomized into the following three groups: the CLP model group (CLP group, n = 11), the NRG-1-treated group (NRG group, n = 11), and the sham-operated group (SHAM group, n = 5). 30 min before the surgery, the rats of the NRG group were injected in the tail vein with recombinant human NRG-1 diluted with normal saline (rhNRG-1, protein purity ≥ 95%, Lot: LC08SE2815 Sino Biological Inc., China) at a dose of 10 μg/kg, whereas those of the other two groups were treated with the same volume of sterile normal saline.

2.3. Sepsis model

Animals were anesthetized with 2% sodium pentobarbital in saline (40 mg/kg, intraperitoneally; Sigma Chemical Co., USA) and placed on a warming pad (37 °C). Cecal ligation and puncture (CLP) was performed as described in a previous study (Rittirsch et al., 2009). Briefly, the rats' abdomens were shaved and a 2-cm-long midline abdominal incision was made. The cecum was carefully isolated and ligated at about 60% of the total length just below the ileocecal valve to prevent bowel obstruction. On the antimesenteric side, two punctures were made with a sterile 18-gauge needle. Then, the cecum was pressed gently to expel a small amount of stool from the puncture site to ensure a full-thickness perforation. The cecum was returned to the peritoneal cavity, and the abdominal incision was closed in two layers. Sham-operated rats underwent the same procedure, but the cecum was neither ligated nor punctured. All rats were resuscitated with subcutaneous injection of normal saline (5 ml/100 g) immediately after surgery. Then they were group-housed in cages with dry sawdust bedding in a temperature-controlled room (22 °C). Soft food and fluid blocks were provided in a tray on the cage floor. The animals were monitored and their status, including behavioral signs and mortality, was recorded every 4 h after the CLP procedure.

2.4. Catheter-based hemodynamic measurements and specimen collection

24 h after surgery, the rats were again anesthetized with 2% sodium pentobarbital in saline (40 mg/kg, intraperitoneally) and placed on a warming pad (37 °C). After tracheal intubation, the rats were placed on positive-pressure ventilation using a rodent ventilator with a respiratory rate of 70 and a tidal volume 6 times the animal's weight. After exposing the right carotid artery, a catheter was inserted into the right common carotid artery to quantify the arterial blood pressure with a BIOPAC MP150 system. After measuring the arterial blood pressure (mean arterial pressure (MAP)), the catheter was introduced into the left ventricle via the right carotid artery to monitor the left ventricular pressure (LVP), LV +dp/dt max (max rate of pressure rise), and LV −dp/dt max (max rate of pressure decay) (Huang et al., 2009). At the end of the experiment, blood samples were drawn from the right carotid artery before rats were killed and then centrifuged. The serum samples were stored at −80 °C until the assay. The heart was removed, drained of blood, and then divided into two halves: one used for histopathological examination and the other snap-frozen for subsequent detection.

2.5. Histopathology

The heart tissues were fixed in 4% formalin. After fixation, each tissue sample was processed routinely and embedded in paraffin. Then, 5-μm sections were taken from the tissue blocks and stained with hematoxylin and eosin (H&E). These sections were photographed under a light microscope for histopathological examination. The slides were graded according to inflammatory changes, as described elsewhere (Wang et al., 2008), by a single investigator blinded to the group assignment. A zero score indicated no or questionable presence of lesions in each category. A score of 1+ indicated limited focal distribution of myocardial lesions. Scores ranging from 2+ to 3+ indicated intermediate severity with multiple lesions, whereas a 4+ score indicated extensive lesions over the entire examined heart tissue.

2.6. Enzyme-linked immunosorbent assay

The serum levels of cardiac troponin-T (cTn-T), tumor necrosis factor (TNF-α), IL-1β, and IL-6, and the concentration of macrophage inhibitory factor (MIF) and angiotensin II (Ang II) in the myocardium were measured using enzyme-linked immunosorbent assay (ELISA) kits (Elabscience Biotechnology Co., Ltd, Wuhan, China) according to the manufacturer's instructions (Ebong et al., 1999).