

Original Article

Interleukin 6 increases dysfunction of organs in sepsis rats through sirtuin 1

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Abstract: Sepsis-induced organ failure is the major cause of death, and is characterized by a massive dysregulated inflammatory response. The present study was to determine whether interleukin 6 (IL-6) expression was increased in sepsis rats and the roles of IL-6 in the damage of cardiac, liver and renal function in the sepsis rats. Sepsis rat models were elicited by intravenous injection of LPS. The mRNA and protein of IL-6 levels were increased in the sepsis rats. The Left ventricular developed pressure (LVDP) and average \pm dP/dt were significantly reduced in sepsis rats compare with sham group. ALT and AST activities and creatinine level were increased in the sepsis rats. IL-6 significantly reduced LVDP and average \pm dP/dt, increased the activities of ALT and AST, and increased the concentration of creatinine in the sepsis rats. EX527, a sirtuin 1 (SIRT 1) inhibitor, blocked the effects of IL-6 in the sepsis rats. These results indicate that IL-6 plays important roles in the damage of cardiac, liver and renal function in the sepsis rats through SIRT 1.

Keywords: Interleukin 6, sepsis, cardiac, liver, renal, sirtuin 1

Introduction

Sepsis is the leading cause of death among critically ill patients in intensive care units, and treatment options are limited. Over 70% of patients who develop sepsis-induced multiple organ failure [1]. Multiple organ failure is a leading cause of mortality in sepsis, and myocardial depression is the most common organ dysfunction including decreased myocardial contractility, impaired ventricular response to fluid therapy and ventricular dilatation [2]. The hallmark of sepsis is a dysregulated and overwhelming inflammatory response, characterized by oxidative stress, mitochondrial dysfunction, and massive cytokine release, which caused cellular damage and organ dysfunction [3-6].

The development of metabolic acidosis in patients with sepsis and septic shock is often accompanied by impairment in organ function and an increase in morbidity and mortality [7]. Sepsis induced by cecal ligation and puncture (CLP) resulted in a severe impairment of the indices of cardiovascular performance includ-

ing decrease in mean arterial pressure, left ventricle systolic pressure and \pm dP/dt max, and increase in left ventricle end-diastolic pressure [8, 9]. Pre-existing liver dysfunction is a risk factor for the progression of infection to sepsis. Liver dysfunction after sepsis is an independent risk factor for multiple organ failure and sepsis-induced death [10]. It has been shown that, in critically ill patients, acute kidney injury (AKI) commonly occurs in association with sepsis and its presence portends an increased likelihood of poor outcomes [11]. However, the molecular mechanisms of sepsis induced organ function damage are not very clear.

Interleukin 6 (IL-6) is a proinflammatory cytokine produced during infections and is well-known predictors of blood culture positivity in patients with sepsis [12]. Resveratrol inhibits LPS-induced inflammation via sirtuin 1 (SIRT 1), and indicated that SIRT 1 is an efficient target for the regulation of LPS-induced inflammation [13]. However, the precise relationship between IL-6 and sepsis and the molecular mechanisms of IL-6 in the sepsis are not well understood.

Interleukin 6 in the sepsis rats

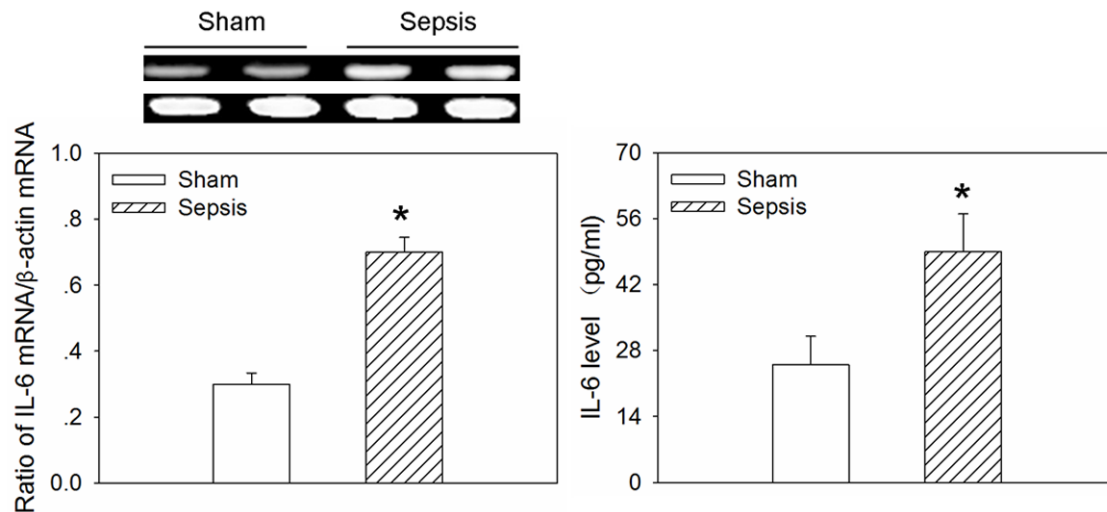


Figure 1. IL-6 mRNA expression and IL-6 protein level in the plasma in the rats with sepsis. Values are mean \pm SE. * $P < 0.05$ versus Sham. $n = 8$ for each group.

The present study was designed to determine the roles of IL-6 in the damage of cardiac, liver and renal function in the sepsis rats.

Materials and methods

Animals and sepsis rat model

Male Sprague-Dawley rats weighing 250-300 g were purchased from the Chinese Academy of Medical Sciences Laboratory Animal Center. The rats were kept in a temperature-controlled room on a 12 h-12 h light-dark cycle with free access to standard chow and water. To establish the sepsis rat model, each rat was anaesthetized with urethane (800 mg kg⁻¹, I.P.) and α -chloralose (40 mg kg⁻¹, I.P.). Adequate depth of anesthesia was assessed by the absence of corneal reflexes and paw withdrawal response to a noxious pinch. After intraperitoneal anesthesia, each rat was injected of LPS 10 mg/kg (intravenous infusion for 10 min) [14]. Normotensive sham-operated (Sham) rats received saline injection.

Reverse transcriptase (RT)-PCR

The mRNA expressions of IL-6 were detected by RT-PCR. Especially, the primers were designed as follows: forward primer: 5'-GACAGCCACTCACCTCTTCA-3', and reverse primer: 5'-AGTGCCTCTTTGCTGCTTTC-3'; for β -actin, forward primer, 5'-GGATGCAGAAGGAGATCACTG-3', and reverse primer, 5'-CGATCCACACGGAGTACTTG-3'.

The level of REG4 mRNA was normalized to the β -actin mRNA level.

Measurement of IL-6 level

IL-6 in the plasma was measured using an enzyme-linked immunoassay kit (R&D Systems, Minneapolis, MN) following the manufacturer's instructions. Briefly, a 96-well microplate was coated with an antibody specific for rat IL-6. We added 100 μ l of sample and 100 μ l of standard diluent buffer to each well in duplicate, incubated it for 90 min at 37°C, and then washed it five times. Subsequently, 100 μ l of biotinylated anti-IL-6 antibody solution were added, incubated for 60 min at 37°C, and then washed. 100 μ l of streptavidin-horseradish peroxidase conjugate solution were added, incubated for 30 min at 37°C, and washed. 100 μ l of chromagen solution were added and incubated in the dark for 15 min at 37°C. The reaction was stopped and read at 450 nm using an ELISA plate reader. Standardization curves were made with known concentrations of rat IL-6.

Left ventricular pressure recording

The preparation of heart isolation and measurement of cardiac contractility were performed as described previously [15]. Hearts were isolated 6 h after LPS administration and mounted on the Langendorff apparatus (ML7-85B2 Langendorff System Bundle, AD instruments). The left ventricular developed pressure

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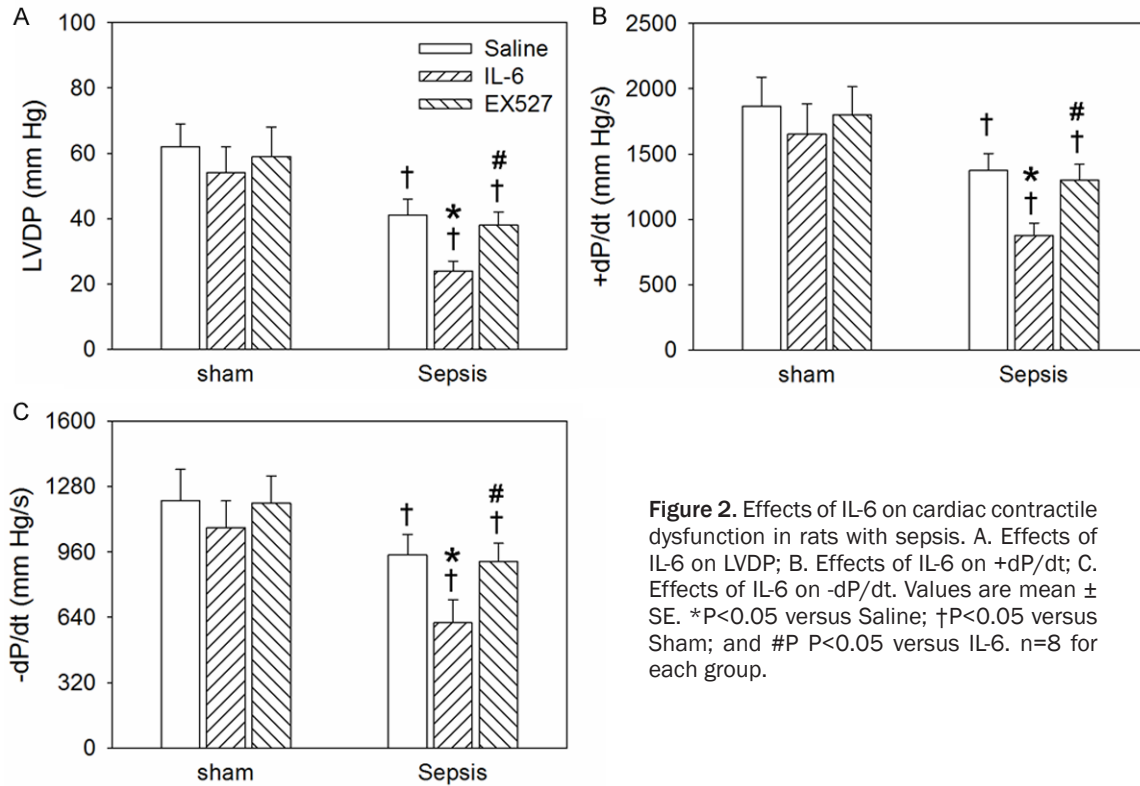


Figure 2. Effects of IL-6 on cardiac contractile dysfunction in rats with sepsis. A. Effects of IL-6 on LVDP; B. Effects of IL-6 on +dP/dt; C. Effects of IL-6 on -dP/dt. Values are mean \pm SE. *P<0.05 versus Saline; †P<0.05 versus Sham; and #P P<0.05 versus IL-6. n=8 for each group.

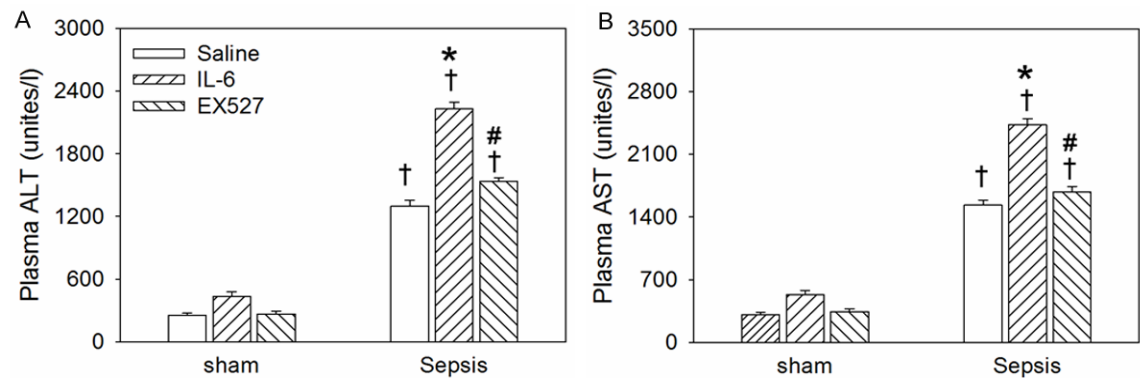


Figure 3. Effects of IL-6 on plasma levels of (A) alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST) in rats with sepsis. Values are mean \pm SE. *P<0.05 versus Saline; †P<0.05 versus Sham; and #P P<0.05 versus IL-6. n=8 for each group.

(LVDP) and the mean rates of contraction (+dP/dt) and relaxation (-dP/dt) were measured.

Liver and renal function

Hepatocellular damage was assessed using plasma alanine amino transferase (ALT) and aspartate amino transferase (AST) activity. Renal function was as assessed using plasma creatinine concentration. Plasma samples were assayed in duplicate on an Advia 2400

Chemistry System (Siemens Healthcare Diagnostics, Deerfield, IL, USA). ALT and AST were measured enzymatically [16] and creatinine was measured colorimetrically [17].

Results

IL-6 mRNA and protein level

The expression of the IL-6 mRNA was increased in plasma in the rats with sepsis; IL-6 protein

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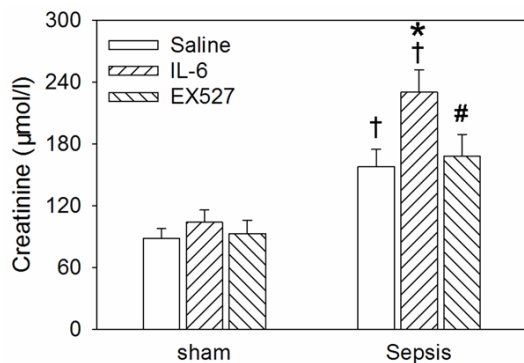


Figure 4. Effects of IL-6 on plasma levels of creatinine in rats with sepsis. Values are mean \pm SE. * $P < 0.05$ versus Saline; † $P < 0.05$ versus Sham; and # $P < 0.05$ versus IL-6. $n = 8$ for each group.

expression was also increased in the rats with sepsis (**Figure 1**).

Effects of IL-6 on cardiac contractile dysfunction in sepsis rats

The LVDP and average $\pm dP/dt$ were significantly reduced in sepsis rats induced by LPS injection compare with sham group. IL-6 significantly reduced LVDP and average $\pm dP/dt$ in the sepsis rats. However, IL-6 has no significant effects on the LVDP and average $\pm dP/dt$ in the sham rats. EX527, a SIRT 1 inhibitor, blocked the effects of IL-6 on the average $\pm dP/dt$ and LVDP reduce (**Figure 2**).

Effects of IL-6 on liver dysfunction in sepsis rats

Plasma ALT and AST activities were increased in the sepsis rats. IL-6 significantly increased the activities of ALT and AST in the rats with sepsis but has no effect of ALT and AST activities in the sham rats. SIRT 1 inhibitor EX527 blocked the effects of IL-6 on the activities of ALT and AST increase (**Figure 3**).

Effects of IL-6 on renal dysfunction in sepsis rats

Creatinine level was increased in the sepsis rats compared with the sham rats. IL-6 significantly increased the concentration of creatinine in the sepsis rats. EX527 blocked the effects of IL-6 on the creatinine level increase (**Figure 4**).

Discussion

Sepsis remains a leading cause of death in critically ill patients, despite efforts to improve

patient outcome. Sepsis is an important cause of mortality in newborns and life-threatening disorder in infants [18]. The inflammasome is essential to the innate immune response to infection and also important in sepsis induced apoptosis [19]. It has been shown that IL-1A-889, IL-1B+3954 and IL-1RN VNTR might be associated with sepsis susceptibility [20]. In the present study, we demonstrated that the mRNA and protein of IL-6 levels were increased in the sepsis rats. IL-6 plays important roles in the damage of cardiac, liver and renal function in the sepsis rats.

Sepsis can cause myocardial dysfunction, which contributes to the high mortality of sepsis. Mean arterial blood pressure significantly decreased after LPS challenge, and septic shock was observed at the end of experiment [14]. Colon ascendens stent peritonitis (CASP) led to continuous release of bacteria into the peritoneal cavity, an increase of cytokines, and differential regulation of receptors of innate immunity in the heart in the sepsis [21]. Burn plus sepsis injury was associated with a significant deterioration of global hemodynamic and cardiac contractile function and this dysfunction was attenuated by IL-6 deficiency [22]. In the present study, we showed that The LVDP and average $\pm dP/dt$ were significantly reduced in sepsis rats induced by LPS injection compare with sham group. IL-6 significantly reduced LVDP and average $\pm dP/dt$ in the sepsis rats. However, IL-6 has no significant effects on the LVDP and average $\pm dP/dt$ in the sham rats. EX527, a SIRT 1 inhibitor, blocked the effects of IL-6 on the average $\pm dP/dt$ and LVDP reduce. These results showed that IL-6 plays an important role in the function of the cardiac in the sepsis rats through SIRT 1.

It has been shown that Liver dysfunction after sepsis is an independent risk factor for multiple organ dysfunction and sepsis-induced death. The liver works as a lymphoid organ in response to sepsis. Acting as a double-edged sword in sepsis, the liver-mediated immune response is responsible for clearing bacteria and toxins but also causes inflammation, immunosuppression, and organ damage [10]. LPS-induced kidney injury which leads to creatinine level increased in LPS-treated rats [23]. In the present study, we demonstrated that plasma ALT and AST activities were increased in the sepsis rats. IL-6 significantly increased the activities of

ALT and AST, and creatinine concentration in the rats with sepsis but has no effect in the sham rats. SIRT 1 inhibitor EX527 blocked the effects of IL-6 on the activities of ALT and AST, and creatinine level increase. These results showed IL-6 plays important roles in the damage of liver and renal function in the sepsis rats via SIRT 1.

In conclusion, cardiac, liver and renal function was damage. IL-6 plays important roles in the damage of organs function in the sepsis rats.

Disclosure of conflict of interest

None.

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