Biomedicine & Pharmacotherapy 94 (2017) 705–711

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

An experimental study of the protective effect of simvastatin on sepsis-induced myocardial depression in rats

Wang Yu*, Zhang Lichun, Zhao Xin, Yang Wenping, Zhang Rong

Emergency Department, Shengjing Hospital of China Medical university, No. 36 Sanhao street, Heping district, Shenyang, Liaoning Province 110004, China

ARTICLE INFO

Article history:
Received 9 June 2017
Received in revised form 13 July 2017
Accepted 20 July 2017

Keywords:
Sepsis
Simvastatin
Toll-like receptor
NF-κB
Inflammatory mediators
Myocardial protection

ABSTRACT

Many patients with sepsis died of heart failure caused by sepsis-induced myocardial depression. Patients with cardiovascular diseases treated by statins have a lower incidence and mortality of sepsis, although the mechanisms remain elusive. To investigate the protective effect of simvastatin on sepsis-induced myocardial depression and to explore possible mechanisms of action. Thirty six adult male Wistar rats were pretreated with simvastatin (0.2 μg/g, q12 h) for one week before cecal ligation and puncture (CLP). It was found that in simvastatin-treated rats, cardiac function indices, including left ventricular systolic pressure (LVESP) and maximal rate of rise and fall of left ventricular pressure (± dp/dtmax) and mean arterial pressure(MAP) markedly improved. Myocardial cells examined with hematoxylin and eosin (HE) were only partially swollen and degenerated and with fewer inflammatory cells infiltrating. Expressions of TLR4 and NF-κB p65 protein were significantly lower in simvastatin-treated rats than that in sepsis rats at the same time point. Levels of TNF-α, IL-1β, IL-6, MCP-1 and NO in myocardial tissues, together with levels of CTnI in serum were significantly declined in simvastatin-treated rats. Simvastatin has a protective effect on myocardial depression caused by sepsis. The effect may be mediated by the inhibition of TLR4-NF-κB signaling pathway, which leads to reduced levels of downstream inflammatory factors such as TNF-α, IL-1β, IL-6, MCP-1 and NO.

1. Introduction

Sepsis, which is an out of control host response to infection, causes life-threatening organ dysfunction and is a major cause of death in intensive care units. Many patients with sepsis die of heart failure caused by sepsis-induced myocardial depression (SIMD). About 50% of patients with severe sepsis develop some degree of myocardial depression and the mortality of these patients is as high as 70%–90% [1]. Septic myocardial depression may present as decreased myocardial contractility, decreased left ventricular ejection fraction, peripheral vasodilatation and reversible biventricular dilatation [2]. The pathogenesis of SIMD is complex. Recent studies suggest that toll-like receptor 4, an important mediator in innate immune and inflammatory responses, plays a critical role in myocardial depression caused by sepsis [3,4]. Toll-like receptor 4 selectively recognizes bacterial endotoxin E and Gram-negative bacterial lipopolysaccharide (LPS) or lipooligosaccharide [5]. Nuclear factor-κB (NF-κB) is activated by TLR4 and promotes the expression of various cytokines, thus exacerbating myocardial depression. As well as anti-infective treatment, preventing and treating secondary cardiac dysfunction is, therefore, important to reduce sepsis mortality.

Recent studies have shown that, as well as lowering blood lipids, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have anti-inflammatory and immunoregulatory effects and improve endothelial cell functions [6]. Epidemiological studies have suggested that cardiovascular patients who are treated with statins have a lower incidence and mortality of sepsis [7]. However, the molecular mechanisms underlying the protective effects of statins against sepsis-induced cardiac dysfunction remain poorly defined. In the present study, we have established a rat model of sepsis using cecal ligation and puncture (CLP) and investigated the protective effect of simvastatin on SIMD. We also suggest likely mechanisms for the protective effect of simvastatin.

2. Materials and methods

2.1. Experimental animals

Healthy, specific pathogen-free, adult male Wistar rats (120 ± 20 g) were purchased from Charles River Laboratories (Beijing, China) and had an Animal Quarantine Certificate of

* Corresponding author.
E-mail address: wangy8@sj-hospital.org (Y. Wang).

http://dx.doi.org/10.1016/j.biopha.2017.07.105
0753-3322/

Conformity (SCXK (Jing) 2014–0004). All experimental procedures were performed in accordance with the legislation on the protection of animals, and were approved by the Local Ethical Committee for Animal Experimentation (Ethics number: 2015PS251 K).

2.2. Rat sepsis model

Polymicrobial sepsis was induced in rats by CLP, as previously described in detail [8]. Briefly, the animals were anesthetized by intraperitoneal injection of 5% chloral hydrate (0.6 mL/100 g) and a midline incision was made to expose the cecum. The cecum was filled with feces by milking the stool backwards from the ascending colon and 50% of the cecum was ligated with a 5-0 silk suture. The cecum was soaked with phosphate-buffered saline (PBS) (pH 7.4) and was then punctured twice with an 18-gauge needle on the antimesenteric border. Return the cecum to the peritoneal cavity, and the abdominal incision was sutured. Sham-operated rats underwent identical laparotomy and resuscitation procedures, but the cecum was neither ligated nor punctured. The severity of abdominal infection in sepsis rat was assessed using Simon celiac infection classification criteria [9].

2.3. Animal groups

Thirty six Wistar rats were randomly divided into three groups: a sham-operated group, a CLP (sepsis) group and a CLP + simvastatin (treatment) group, with 12 rats in each group. Simvastatin (Melone Pharmaceutical Co., Ltd, Dalian, China) was dissolved in EtOH (10 mg/mL) and the solution was then diluted with 0.9% aqueous NaCl (1:1000), to provide the dosing solution (10 μg/mL). The solution was administered by intraperitoneal injection (0.2 μg/g; injected volume 0.02 mL/g, q12 h) for 7 days before CLP. The sham-operated and sepsis groups received intraperitoneal injections of vehicle (0.02 mL/g, q12 h), prepared as above but without simvastatin.

2.4. Monitoring of heart function

The animals were anesthetized by intraperitoneal injection of 5% chloral hydrate (0.6 mL/100 g) 6, 12 and 24 h after CLP. After exposing the right carotid artery, a catheter was inserted into the right common carotid artery to quantify the mean arterial pressure (MAP) with an RM6240BD multichannel physiological signal acquisition and processing system (Chengdu Instrument Factory, Chengdu, China). After measuring the MAP, the catheter was introduced into the left ventricle via the right carotid artery to monitor cardiac function indices, including left ventricular systolic pressure (LVESP) and maximal rate of rise and fall of left ventricular pressure (± dp/dtmax). Blood samples were collected from the carotid artery and centrifuged immediately (1509*g*, 4 °C, 10 min). The supernatants were collected and stored at −20 °C. When the blood sampling was complete, a thoracotomy was performed and a sample of left ventricular myocardial tissue was removed for future use.

2.5. Histopathological examination of myocardial tissue

Rat myocardial tissues were fixed with 4% paraformaldehyde and the specimens were sliced into tissue blocks with a thickness of ~0.5 cm. Conventional gradient alcohol dehydration, paraffin-embedding, serial sectioning and hematoxylin and eosin (H&E) staining were carried out and histopathological changes in myocardial tissue were observed under an optical microscope. The slides were graded according to inflammatory changes, as described elsewhere [10]. 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 (Table 1).

2.6. Detection of toll-like receptor 4 and NF-κB p65 protein expression in myocardial tissue by western blot

Cytoplasmic and nuclear proteins from myocardial tissue were extracted using appropriate kits (Nanjing Keygen Biotech. Co., Ltd., Nanjing, China), according to the manufacturer's instructions. Protein concentrations were determined using a bicinchoninic acid assay (BCA) kit (Beyotime Institute of Biotechnology, Haimen, China). Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked with non-fat milk and then incubated with primary antibodies (rabbit anti- toll-like receptor 4 or polyclonal anti-NF-κB p65) (Santa Cruz Biotechnology Inc, Texas, USA) at 4 °C overnight. The membranes were then incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:1000, Beyotime Institute of Biotechnology) at 37 °C for 45 min. An enhanced chemiluminescence (ECL) imaging system was used to visualize protein bands. β-actin was used as the internal control.

2.7. Detection of myocardial TNF-α, IL-1β, IL-6, MCP-1, NO and serum cardiac troponin I (CTnI)

Myocardial tissues were homogenized in PBS buffer with a portable high-speed homogenizer (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China). The homogenate was subjected to cyclic freeze-thawing using liquid nitrogen to generate a 10% tissue homogenate, which was then centrifuged for 10 min at 10010*g*. The supernatant was collected and stored at −20 °C for future use. Enzyme-linked immunosorbent assay(ELISA) kits (R&D Systems Inc., Minneapolis, USA) were used according to the manufacturer's instructions to detect myocardial TNF-α,IL-1β, IL-6, MCP-1 and serum CTnI. Absorbance values were read with a microplate reader. NO levels in myocardial tissue were measured using a nitrite/nitrate assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

2.8. Statistical analyses

SPSS Statistics 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Numeric data are presented as mean ± SD. One-way ANOVA was used to compare the means of the three groups and an LSD-t test was used for pairwise comparison between two groups. A P value <0.05 was considered to be statistically significant.

Table 1
Histopathological scores of myocardial tissue.

	6 h	12 h	24 h
Sham-operated group	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sepsis group	2.11 ± 0.17[#]	2.64 ± 0.15[#]	3.12 ± 0.21[#]
Treatment group	1.22 ± 0.08[#,*]	1.56 ± 0.09[#,*]	1.91 ± 0.14[#,*]

Histopathological scores of myocardial tissue in different groups at different time point. Myocardial histological scores ranged from 0 to 4+ for inflammation. Data are presented as the mean ± standard deviation.

[#] $P < 0.05$ compared with the sham group.

[*] $P < 0.05$ compared with the sepsis group.