Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

SRT1720, a sirtuin 1 activator, attenuates organ injury and inflammation in sepsis

Adam Khader, MD, PhD,[a,b] *Weng-Lang Yang, PhD,*[a,b,c] *Laura W. Hansen, MD,*[b] *Salil R. Rajayer, MD,*[c] *Jose M. Prince, MD,*[b,c] *Jeffrey M. Nicastro, MD,*[b] *Gene F. Coppa, MD,*[b] *and Ping Wang, MD*[a,b,c,*]

[a] *Elmezzi Graduate School of Molecular Medicine, Manhasset, New York*
[b] *Department of Surgery, Hofstra Northwell School of Medicine, Hempstead, New York*
[c] *Center for Immunology and Inflammation, The Feinstein Institute for Medical Research, Manhasset, New York*

ARTICLE INFO

Article history:
Received 5 April 2017
Received in revised form
10 May 2017
Accepted 15 June 2017
Available online xxx

Keywords:
SRT1720
Sirtuin 1
Organ injury
Inflammation
Inflammasome
Sepsis

ABSTRACT

Background: Sepsis affects 800,000 patients in the United States annually with a mortality rate of up to 30%. Recent studies suggest that sepsis-associated metabolic derangements due to hypoxic tissue injury, impaired oxygen utilization, and mitochondrial dysfunction contribute to mortality. Sirtuin 1 (Sirt1) is a crucial modulator of energy metabolism during starvation states and has anti-inflammatory effects. Here, we hypothesized that SRT1720, a Sirt1 activator, could attenuate the severity of sepsis.

Materials and methods: Male C57BL/6 mice (20-25 g) were subjected to cecal ligation and puncture (CLP) to induce sepsis. SRT1720 (5 or 20 mg/kg BW) or 10% dimethyl sulfoxide (vehicle) in 0.2-mL saline was injected intravenously at 5 h after CLP. Control animals were not subjected to any surgery. Blood and liver samples were harvested at 20 h after CLP for analysis.

Results: Administration of SRT1720 markedly reduced the serum levels of tissue injury markers (aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase) and renal injury markers (blood urea nitrogen and creatinine) in a dose-dependent manner after CLP. Furthermore, the levels of proinflammatory cytokines interleukin (IL)-1β and IL-6 in the serum and liver were significantly inhibited by SRT1720 treatment after CLP. SRT1720 treatment resulted in a significantly decreased mRNA expression of inflammasome components (nucleotide oligomerization domain–like receptor protein 3, adapter apoptosis-associated speck-like protein containing caspase-recruitment domain, IL-1β, and IL-18) in the liver, compared with the vehicle group.

Conclusions: SRT1720 treatment attenuates multiorgan injury in septic mice. SRT1720 treatment also decreases the production of proinflammatory cytokines and reduces inflammasome activation. Thus, pharmacologic stimulation of Sirt1 may present a promising therapeutic strategy for sepsis.

* *Corresponding author*. Center for Immunology and Inflammation, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030. Tel.: +1 516 562 3411; fax: +1 516 562 1022.
E-mail address: pwang@northwell.edu (P. Wang).
0022-4804/$ – see front matter © 2017 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2017.06.031

Introduction

Sepsis affects an estimated 800,000 patients in the United States annually and carries a mortality rate of up to 30%, despite recent advances in clinical interventions.[1,2] The pathophysiology of sepsis is complex and has focused over the past few decades on the "cytokine theory," which postulates that a host's exaggerated immune response to severe infection increases mortality.[3] Multiple clinical trials have attempted to dampen the host's immune response,[4] most recently with the use of a TLR4 antagonist.[5] However, such attempts have failed to show a mortality benefit in human studies. Thus, further investigations must identify new targets to improve our treatment of sepsis.

In addition to the deleterious hyperinflammatory response in sepsis, metabolic derangement also contributes to overall mortality in sepsis and develops from hypoxic tissue injury, impaired oxygen utilization, and mitochondrial dysfunction.[6,7] These alterations in cellular metabolism ultimately result in a reduction of ATP levels in septic animals and patients.[6] The incidence of hypermetabolism in the early phase of severe sepsis patients is associated with high mortality and poor outcome.[8] Through controlling the energy metabolism pathways, it has been shown to have beneficial effects in the animal model of sepsis.[9,10]

Sirtuin 1 (Sirt1) is an NAD^+-dependent histone deacetylase which primarily modulates energy metabolism by inducing catabolic processes. This activity increases cellular energy stores and maintains energy homeostasis.[11] Activation of Sirt1 has been demonstrated to improve metabolism in murine models of cardiac, renal, intestinal, and hepatic ischemia-reperfusion injury.[12-15] In addition to its role in metabolic modulation, Sirt1 has also played a direct role in regulating inflammation,[16] such as by modulating the activity of nuclear factor kappa B (NF-κB), a critical transcription factor in the regulation of proinflammatory cytokine production.[17]

Several sirtuin activating compounds (STACs) have been developed to investigate stimulating Sirt1 to treat chronic metabolic diseases.[18] STAC treatment before and through sepsis induction has shown beneficial effects.[19] However, the effect of STAC treatment after sepsis induction, which more closely resembles clinical therapy, has not been evaluated. SRT1720 is identified as one of the most potent STACs.[18] In this study, we further explored the interplay between energy metabolism and the hyperinflammatory phase of sepsis. We determined the effect of SRT1720 after treatment on organ injury and inflammation in mice undergoing cecal ligation and puncture (CLP), a clinically relevant model that induces polymicrobial sepsis.[20] Recently, the formation of inflammasome complexes has also been identified as a pathway to release proinflammatory cytokines during microbial infections.[21] Thus, we also examined the effect of SRT1720 treatment on the expression of several inflammasome proteins in the liver of septic mice.

Materials and methods

Animal model of sepsis

Male C57BL/6 mice (20-25 g) were obtained from Charles River Laboratories (Wilmington, MA). Only males were used in this study to eliminate potential gender variability. Septic peritonitis was induced by CLP. Animals were anesthetized with inhaled isoflurane. The peritoneal cavity was opened and the cecum was ligated immediately distal to the ileocecal valve with 4-0 silk suture. The cecum was then punctured through and through with a 22-gauge needle, and a small amount of fecal material was expressed. The cecum was then returned to the peritoneal cavity, and the midline incision was closed in two layers with 4-0 nylon suture. All animals were resuscitated with 1 mL of subcutaneous saline and were returned to their cages with food and water *ad lib*. By following this procedure, the 10-d survival rate is 26% as shown in our recent publication.[22] All experimental procedures were approved by the Institutional Animal Care and Use Committee of The Feinstein Institute for Medical Research (protocol #2013-002) and were in accordance with the guidelines for the use of experimental animals by the National Institutes of Health (Bethesda, MD).

Administration of SRT1720

Five hours after CLP, animals were injected intravenously with either 5 or 20 mg/kg body weight of SRT1720 (*N*-[2-[3-(piperazin-1-ylmethyl)imidazo[2,1-b][1,3]thiazol-6-yl]phenyl] quinoxaline-2-carboxamide, ADOOQ Bioscience, Irvine, CA) or 10% dimethyl sulfoxide in normal saline (vehicle). Based on our previous study, significant organ injury can be detected in mice 20 h after CLP.[22] Thus, at 20 h after CLP, animals were sacrificed for collection of blood and liver, which were stored in −80°C before analysis. Control animals underwent no intervention.

Assessment of serum organ injury markers

Serum was collected by centrifuging blood samples at 7000 rpm for 10 min, after which the serum was stored at −80°C. Activity levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were measured by commercial assays from Pointe Scientific (Lincoln Park, MI). Similarly, serum levels of creatinine and blood urea nitrogen (BUN) were determined by commercial kits from the same company. Both assays were performed per the instructions provided by the manufacturer.

Measurement of cytokine levels

The liver tissue was homogenized and lyzed using a sonic dismembrator in lysis buffer (10-mM Tris-buffered saline at pH 7.5 with 1% Triton-X 100, 1-mM ethylenediaminetetraacetic acid, and 1-mM ethylene glycol tetraacetic acid) containing a protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). Interleukin (IL)-1β and IL-6 levels in serum and liver lysates were quantified by enzyme-linked immunosorbent assay (ELISA) kits from BD Biosciences (San Diego,