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SRT1720, a Sirtuin 1 Activator, Attenuates Organ Injury and Inflammation in Sepsis

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Abstract

Background—Sepsis affects 800,000 patients in the US 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 (20–25g) 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 (AST, ALT, and LDH) and renal injury markers (BUN and creatinine) in a dose-dependent manner after CLP. Furthermore, the levels of proinflammatory cytokines IL-1 β 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 (NLRP3), adapter apoptosis-associated speck-like protein containing caspase-recruitment domain (ASC), IL-1 β , and IL-18] in the liver, compared to the vehicle group.

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Conclusions—SRT1720 treatment attenuates multi-organ 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.

Keywords

SRT1720; Sirtuin 1; Organ injury; Inflammation; Inflammasome; Sepsis

Introduction

Sepsis affects an estimated 800,000 patients in the US 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 hyper-inflammatory 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- κ B), 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 effects¹⁹. 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 hyper-inflammatory phase of sepsis. We determined the effect of SRT1720 post-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 (20-25g) 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 cecal ligation and puncture (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-day 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 (DMSO) 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 prior to analysis. Control animals underwent no intervention.

Assessment of serum organ injury markers

Serum was collected by centrifuging blood samples at 7,000 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 lysed using a sonic dismembrator in lysis buffer (10 mM TBS at pH 7.5 with 1% Triton-X 100, 1 mM EDTA, and 1 mM EGTA) containing a protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). Interleukin 1 beta (IL-1 β) and IL-6 levels in serum and liver lysates were quantified by enzyme-linked immunosorbent assay (ELISA) kits from BD Biosciences (San Diego, CA). The assay was performed per the instructions provided by the manufacturer.

Determination of mRNA levels by quantitative PCR

Total RNA was extracted from liver tissue using Trizol (Invitrogen, Carlsbad, CA). The RNA was then reverse-transcribed into complementary DNA using murine leukemia virus reverse transcriptase (Applied Biosystems, Foster City, CA). PCR was performed using the complementary DNA and DEPC water, 12.5 μ L SYBR Green PCR Master Mix (Applied Biosystems), and 0.08 μ mol each of forward and reverse primers, for a total reaction volume of 25 μ L. The amplification was performed in an Applied Biosystems 7300 real-time PCR machine using the following protocol: 50°C for 2 min, 95°C for 10 min, and 45 cycles of 95°C for 15 sec and 60°C for 1 min. Mouse β -actin was used for normalization. Relative expression of mRNA was calculated as fold change in comparison with control levels. Primers are listed in Table 1.

Statistical analysis

All data are expressed as a mean $\pm$ standard error and compared by one-way analysis of variance using the Student-Newman-Keuls test. Student's *t*-test was used for two-group analysis. Differences in values were considered significant at $P < 0.05$.

Results

ST1720 treatment reduces organ injury after CLP

The severity of sepsis-induced by CLP was determined by measuring the serum activities of organ injury markers, AST, ALT, and LDH. At 20 h after CLP, AST, ALT, and LDH were increased by 14-, 6-, and 203-fold, respectively, compared to the control mice (Fig. 1). These three markers are commonly used to monitor the severity of organ injury and are well-correlated with histological analysis for organ damage.^{23,24} In order to evaluate the effect of post-treatment on septic mice, we administrated SRT1720 at 5 h after CLP, when the mice are in the hyper-inflammatory phase and have significantly increased their expression of pro-inflammatory cytokines.^{25,26} Treatment with 5 and 20 mg/kg SRT1720 at 5 h after induction of sepsis resulted in a significant reduction in AST by 32.4% and 46.5%, respectively, compared to vehicle-treated mice (Fig. 1A). There was a 34.3% reduction of ALT activity with SRT1720 treatment at 20 mg/kg; however, it did not reach statistical significance (Fig. 1B). Treatment with SRT1720 at 5 and 20 mg/kg decreased the LDH levels by 28.5% and 62.8%, respectively, compared to the vehicle-treated group, in a dose-dependent manner (Fig. 1C).

Renal injury is improved by SRT1720 treatment after CLP

Acute kidney injury (AKI) can be induced by a septic insult.²⁷ Serum BUN and creatinine levels were used as indicators of AKI after CLP. Twenty hours after CLP, serum BUN and creatinine were increased by 2.7- and 2.3-fold, respectively, compared to control mice (Fig. 2). Treatment with 5 and 20 mg/kg SRT1720 reduced BUN levels by 22.5% and 32.2%, respectively, compared to vehicle-treated mice (Fig. 2A). There was a reduction in creatinine with SRT1720 treatment only at the 20 mg/kg group, which reached a significant decrease by 41.1% compared to vehicle-treated group (Fig. 2B).

SRT1720 treatment decreases inflammation after CLP

A hyper-inflammatory response is a major factor in contribution to organ injury in sepsis.³ We measured serum and liver levels of proinflammatory cytokines at 20 h after CLP. Serum IL-1 β and IL-6 and protein levels were increased 72- and 1,562-fold, respectively, after induction of sepsis (Fig. 3). Treatment with increasing doses of SRT1720 was associated with a reduction in both cytokines in serum, with the greatest reduction observed at 20 mg/kg to the levels comparable to the control (Fig. 3). In liver tissue lysates, levels of IL-1 β and IL-6 were increased by 2.3- and 5.0-fold, respectively, at 20 h after CLP, compared to the control (Fig. 4). SRT1720 treatment at 5 mg/kg showed a slight decrease in liver IL-1 β and IL-6 levels; however, with treatment at 20 mg/kg, their levels were significantly decreased by 36.8% and 75.1%, respectively, compared to the vehicle-treated group (Fig. 4).

SRT1720 treatment inhibits the expression of inflammasome components in the liver after CLP

Among the various inflammasome complexes, the nucleotide oligomerization domain-like receptor protein 3 (NLRP3) inflammasome is the most studied and can be activated by diverse stimuli.²⁸ When stimulated, NLRP3 forms a complex with the adapter apoptosis-associated speck-like protein containing caspase-recruitment domain (ASC) and pro-caspase-1, followed by releasing proinflammatory cytokines, IL-1 β and IL-18.²⁸ The mRNA levels of NLRP3 and ASC in the liver were increased by 5.8- and 1.8-fold, respectively, at 20 h after induction of sepsis, compared to control mice (Fig. 5A and B). Treatment with SRT1720 decreased NLRP3 mRNA expression in a dose-dependent manner, by 66.7% in the 5 mg/kg group and 83.4% in the 20 mg/kg group, as compared to vehicle-treated mice (Fig. 5A). The mRNA levels of ASC exhibited a significant reduction by 36.7% with SRT1720 treatment at 20 mg/kg compared to vehicle mice (Fig. 5B). Subsequently, the mRNA expressions of IL-1 β and IL-18 in the liver were also increased by 24.9- and 9.6-fold, respectively, at 20 h after CLP, compared to vehicle group (Fig. 5C and D). Treatment with 20 mg/kg SRT1720 was associated with a 73.7% decrease of IL-1 β and a 66.5% decrease of IL-18 mRNA levels when compared to vehicle-treated mice (Fig. 5C and D).

Discussion

Sepsis is a complex medical condition traditionally associated with an acute hyper-inflammatory response followed by late immune suppression.²⁹ As research reveals more about the pathophysiology of sepsis, the importance of the metabolic derangements accompanying this process expands.³⁰ Sirt1 plays a key role in modulating cellular metabolism in starvation states similar to the hypoxic, hypoperfused state of sepsis.¹¹ In this study, we focused on the role of Sirt1 activation in the early phases of sepsis when excessive inflammation predominates. SRT1720 is a very potent and specific small molecule activator of Sirt1.¹⁸ We have demonstrated that administration of SRT1720 at 5 h after induction of sepsis attenuates hepatic and renal injury in septic mice. It also reduced systemic and liver inflammation by inhibiting formation of the inflammasome complex in the liver of septic mice.

In concordance with our observations, several studies also show that activation of Sirt1 had a protective effect in animal models of sepsis. Opal *et. al.* have demonstrated that oral administration of SRT3025, another STAC, significantly improves a 7-day survival of septic mice induced by CLP.¹⁹ Although in their study SRT3025 is given starting 48 h before CLP, we administered SRT1720 at 5 h after CLP. Resveratrol, a less specific Sirt1 activator, reduces AST, ALT, and histologic liver damage in septic rats induced by CLP³¹, and it decreases pulmonary edema and lung pathological alterations in the mouse model of sepsis induced by lipopolysaccharide (LPS).³² Conversely, when a Sirt1 inhibitor (nicotinamide) was used as a pre-treatment before CLP induction, the rats exhibited aggravated damage, oxidative stress, and apoptosis in the liver.³¹ In contrast to a pre-treatment paradigm, administration of a Sirt1 inhibitor (EX-527) at 24 h after CLP induction when these animals are in the hypo-inflammatory phase significantly increased the survival rate of septic mice compared to the untreated septic mice.²⁶ Therefore, the beneficial effect of Sirt1 activation on sepsis may be dependent on the inflammatory status of the animals.³³

The organ injury associated with severe sepsis is often attributed to the acute hyper-inflammatory response evident in early sepsis.³ We have found increased serum and liver levels of proinflammatory cytokines IL-1 β and IL-6 at 20 h after sepsis induction, while post-treatment with SRT1720 significantly reduced the production of these proinflammatory cytokines in septic mice. Activation of Sirt1 associated with the reduced expression of proinflammatory cytokines has also been observed in the septic animals induced by CLP or LPS with treatment of resveratrol.^{31,32} Moreover, stimulation of Sirt1 by SRT3025 reduces the levels of proinflammatory cytokines in the lungs of animals challenged with *S. pneumoniae*.¹⁹ Macrophages are the major source of proinflammatory cytokines after activation. An *in vitro* study demonstrated that enhancing the Sirt1 expression inhibits the secretion of IL-6 and TNF- α in murine macrophages stimulated with LPS.³⁴ The direct anti-inflammatory effect of Sirt1 is through the deacetylation of nf- κ b to suppress its activity.^{17,35} nf- κ b serves as a master transcription factor to regulate the expression of broad range of cytokines.³⁶

Inflammasome formation provides another channel to release proinflammatory cytokines, such as IL-1 β and IL-18.²¹ We have observed an up-regulation of inflammasome-related proteins in the liver of mice after CLP. The activation of hepatic NLRP3 inflammasome has also been reported in rats undergoing burn injury combined with LPS injection.³⁷ We have further demonstrated that SRT1720 treatment inhibits the expression of NLRP3 and ASC, two major protein components involved in forming a inflammasome complex, in the liver of septic mice. We have also observed a reduction of IL-1 β and IL-18 expression in septic mice treated with SRT1720. The involvement of Sirt1 in regulating inflammasome has also been reported in other disease conditions. Administration of resveratrol can inhibit the expression of NLRP3, ASC, and caspase-1 in the liver of the obese mice induced by high-fat diet fed, reducing hepatic metaflammation.³⁸ Similarly, resveratrol treatment decreases the expression of NLRP3 and cleaved IL-1 β in the brain of septic mice.³⁹ Specifically by using inducible Sirt1 knockout mice, Gao *et. al.* have demonstrated increased inflammasome activity in the lung of Sirt1-deficient mice compared to wild-type littermates after CLP.⁴⁰ The transcription activation of inflammasome components like NLRP3 can be regulated by active nf- κ b which in turn is inhibited by Srt1 activation.^{41,42}

Inflammasome can be activated by reactive oxygen species (ROS) and mitochondrial DNA release into the cytosol due to mitochondrial dysfunction and apoptosis.⁴³ It has been reported that the generation of free radicals is increased in the liver of septic animals.³¹ Furthermore, we previously have demonstrated that SRT1720 treatment inhibits oxidative stress, reduces apoptosis, and restores mitochondrial integrity in the liver of mice after hepatic ischemia-reperfusion injury.¹⁵ Upregulation of Sirt1 has also been shown to inhibit apoptosis and protect against oxidative stress, ameliorating hepatic steatosis and acute liver injury.⁴⁴ Therefore, SRT1720 may act through Sirt1 to attenuate the ROS production and maintain mitochondrial homeostasis, leading to downregulation of the of NLRP3 activation in the liver of septic mice. The detail mechanism by which SRT1720 treatment regulates the protein-protein interaction among the inflammasome complex in the liver of septic mice needs further investigation.

To date, SRT1720 has only been studied in animals, and its safety and efficacy in humans has not been established. Although we have demonstrated the protective effects of SRT1720 treatment in septic mice, its long-term beneficial effect and efficacy need further to be determined by a survival study as well as histological analysis of organ damage. Furthermore, only male animals were performed in this study, and whether septic female mice will also benefit from SRT1720 treatment requires further evaluation. Other STACs, like SRT2140, have been in phase II clinical trials for the treatment of patients with moderate to severe psoriasis⁴⁵ and type 2 diabetes.⁴⁶ The majority of adverse events in these clinical trials were mild to moderate in severity and self-limiting. The most frequently reported adverse events in the SRT2104 arms were: dizziness, headache, psoriatic arthropathy, gastrointestinal symptoms and transaminase elevations.^{45,46}

Conclusions

We have found that treatment with SRT1720 ameliorates systemic, liver, and kidney organ injury by reducing systemic and liver inflammation in mice with sepsis from CLP. We have also found that SRT1720 can reduce hepatic inflammasome activation in septic mice. Post-treatment with SRT1720, a specific and potent Sirt1 activator, provides a beneficial effect in the early sepsis response. Thus, pharmacologic Sirt1 activation may represent a potential therapy for polymicrobial sepsis.

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Fig. 1.

SRT1720 decreased serum levels of organ injury markers in mice after CLP. Serum samples were collected at 20 h after CLP to measure (A) AST, (B) ALT, and (C) LDH. Data are presented as means $\pm$ s.e.m. ($n=5-6$ /group). * $P < 0.05$ vs. control; # $P < 0.05$ vs. vehicle. AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase.

Fig. 2.

SRT1720 lowered renal injury in mice after CLP. Serum samples were collected at 20 h after CLP to measure (A) BUN and (B) creatinine. Data are presented as means $\pm$ s.e.m. (n=5-6/group). * P < 0.05 vs. control; # P < 0.05 vs. vehicle. ^ P < 0.05 vs. vehicle (Student's t-test). BUN, blood urea nitrogen.

Fig. 3. SRT1720 attenuated systemic inflammatory cytokine levels in mice after CLP. Serum samples were collected at 20 h after CLP to measure (A) IL-1 β and (B) IL-6 by ELISA. Data are presented as means $\pm$ s.e.m. (n=5-6/group). * $P < 0.05$ vs. control; # $P < 0.05$ vs. vehicle.

Fig. 4. SRT1720 reduced liver inflammatory cytokine levels in mice after CLP. Liver tissue was collected at 20 h after CLP. Tissue lysates were then prepared to measure (A) IL-1 β and (B) IL-6 by ELISA. Data are presented as means $\pm$ s.e.m. (n=5-6/group). * P < 0.05 vs. control; # P < 0.05 vs. vehicle.

Fig. 5.

SRT1720 inhibited the expression of inflammasome molecules in the liver of mice after CLP. The total RNA was isolated from the liver tissue collected at 20 h after CLP. The mRNA levels of (A) NLRP3, (B) ASC, (C) IL-1 β , and (D) IL-18 were measured by quantitative PCR. Each gene expression level was normalized to β -actin. The value in the control group is designated as 1 for comparison. Data are presented as means $\pm$ s.e.m. (n=5-6/group). * P < 0.05 vs. control; # P < 0.05 vs. vehicle; ^ P < 0.05 vs. vehicle (Student's t-test).

Table 1
The list of primers used in this study

Name	Genbank	Forward	Reverse
NLRP3	NM_145827.3	GGGATATCTCTCCCGCATCT	TTGAGGTCCACACTCTCACC
ASC	NM_023258.4	GAGCAGCTGCAAACGACTAA	GTCCACAAAGTGCCTGTTCTG
IL-1 β	NM_008361	CAGGATGAGGACATGAGCACC	CTCTGCAGACTCAAACCTCCAC
IL-18	NM_008360.1	CCTTCCAAATCACTTCCTCTTG	TTGTCTGATTCCAGGTCTCC
β -actin	NM_007393	CGTGAAAAGATGACCCAGATCA	TGGTACGACCAGAGGCATACAG