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Protective effect of suberoylanilide hydroxamic acid against lipopolysaccharide-induced liver damage in rodents

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ARTICLE INFO

Article history:
Received 24 July 2014
Received in revised form
20 October 2014
Accepted 31 October 2014
Available online 6 November 2014

Keywords:
Histone deacetylase inhibitor
LPS
Liver
Inflammation
Apoptosis

ABSTRACT

Background: Lipopolysaccharide (LPS) has a deleterious effect on several organs, including the liver, and eventually leads to endotoxic shock and death. LPS-induced hepatotoxicity is characterized by disturbed intracellular redox balance and excessive reactive oxygen species (ROS) accumulation, leading to liver injury. We have shown that treatment with suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, improves survival in a murine model of LPS-induced shock, but the protective effect of SAHA against liver damage remains unknown. The goal of this study was to investigate the mechanism underlying SAHA action in murine livers.
Method: Male C57BL/6J mice (6–8 wk), weighing 20–25 g, were randomly divided into three groups: (A) a sham group was given isotonic sodium chloride solution (10 μL/g body weight, intraperitoneal, i.p.) with dimethyl sulfoxide (DMSO; 1 μL/g body weight, i.p.); (B) an LPS group was challenged with LPS (20 mg/kg, i.p.) dissolved in isotonic sodium chloride solution with DMSO; (C) and an LPS plus SAHA group was treated with SAHA (50 mg/kg, i.p.) dissolved in DMSO immediately after injection of LPS (20 mg/kg, i.p.). Mice were anesthetized, and their livers were harvested 6 or 24 h after injection to analyze whether SAHA affected production of ROS and activation of apoptotic proteins in the liver cells of challenged mice.
Results: SAHA counteracted LPS-induced production of ROS (thiobarbituric acid reactive substances and nitrite) and reversed an LPS-induced decrease in antioxidant enzyme, glutathione. SAHA also attenuated LPS-induced hepatic apoptosis. Moreover, SAHA inhibited activation of the redox-sensitive kinase, apoptosis signal-regulating kinase-1, and the mitogen-activated protein kinases, p38 and Jun N-terminal kinase.
Conclusions: Our data indicate, for the first time, that SAHA is capable of alleviating LPS-induced hepatotoxicity and suggest that a blockade of the upstream events required for apoptosis signal-regulating kinase-1 action may serve as a new therapeutic option in the treatment of LPS-induced inflammatory conditions.

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0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2014.10.056

1. Introduction

Sepsis, a systemic inflammatory response syndrome capable of inducing endotoxic shock, is a leading cause of mortality in the intensive care unit and has proven to be an exceedingly difficult condition to treat [1]. Infection from gram-negative bacteria is currently the most common cause of sepsis, and lipopolysaccharide (LPS) found on such bacteria is an endotoxin that has been implicated in the pathogenesis of infection and ensuing septic shock [2]. The prooxidant action of LPS induces excessive accumulation of reactive oxygen species (ROS), leading to cellular injury through the impairment of vital macromolecules as well as subsequent altered membrane fluidity and mitochondrial function [3,4]. Growing evidence has demonstrated that ROS are important mediators in caspase-9-dependent apoptotic processes, which have been implicated in a variety of inflammatory and stress signaling pathways [5].

Apoptosis signal-regulating kinase-1 (ASK1) is a key ROS-regulated kinase that controls mitogen-activated protein kinase pathway activation [6]. The inactive form of ASK1 is bound to the reduced form of thioredoxin and 14-3-3 proteins, whereas thioredoxin oxidation and the release of 14-3-3 result in the activation of both ASK1 and p38 kinase [7]. Additionally, LPS-mediated ROS production has previously been reported to activate ASK1, further suggesting that the ROS-dependent ASK1-p38 axis plays a crucial role in LPS-mediated mammalian innate immunity [8,9].

Chromatin structure plays a central role in regulating gene expression and cellular activity. The acetylation of histones is an essential epigenetic mechanism controlling chromatin structure, DNA accessibility for transcription factors, and gene expression. Such protein acetylation is regulated by the opposing actions of histone acetyltransferases and histone deacetylases (HDACs) and affects diverse biological functions, including cell survival [10]. Suberoylanilide hydroxamic acid (SAHA, vorinostat), a potent histone deacetylase inhibitor, has recently emerged as a possible therapeutic intervention for hyperinflammation, as we and others have shown that SAHA reduces the lethality of hemorrhagic shock, suppresses proinflammatory cytokine expression, and improves survival of mice in models of endotoxic shock [10,11]. Recently, we have reported that LPS injection reduces acetylation of proteins, including histone H3K9, H2AK5, and H2BK5, in a murine model of LPS-induced shock. Treatment of mice with SAHA inhibits HDACs, restores the protein acetylation, suppresses proinflammatory cytokine expression, and improves survival [12]. The precise mechanism underlying SAHA-mediated HDAC inhibition, however, remains largely unknown. The aim of the present study was to investigate whether SAHA affects (1) expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), (2) oxidative stress, (3) activation of caspase-3 and -9, (4) liver injury, and (5) phosphorylation of ASK1, p38, and Jun N-terminal kinase (JNK) in a murine model of LPS-induced shock.

2. Materials and methods

2.1. Materials

LPS from *Salmonella typhosa* and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO). SAHA was purchased from Biomol International (Plymouth Meeting, PA). Trizol, SuperScript II Reverse Transcriptase, and Platinum PCR SuperMix were purchased from Life Technologies (Grand Island, NY).

2.2. Mouse protocols

Research was conducted in compliance with the Animal Welfare Act and was approved by the Institutional Animal Care and Use Committee. Male C57BL/6J mice (6–8 wk) weighing 20–25 g were purchased from Jackson Laboratory (Bar Harbor, ME). The mice were randomly divided into three groups as follows: (A) a sham group was given isotonic sodium chloride solution (10 μL/g body weight, intraperitoneal, i.p.) with DMSO (1 μL/g body weight, i.p.); (B) an LPS group was challenged with LPS (20 mg/kg, i.p.) dissolved in isotonic sodium chloride solution with DMSO; (C) and an LPS plus SAHA group was treated with SAHA (50 mg/kg, i.p.) dissolved in DMSO immediately after injection of LPS (20 mg/kg, i.p.). Mice were anesthetized, and their livers were harvested 6 or 24 h after injection and frozen with liquid nitrogen until further use.

2.3. Determination of thiobarbituric acid reactive substances, nitrite, and glutathione

Thiobarbituric acid reactive substances (TBARS), nitrite, and glutathione (GSH) assay kits were purchased from Cayman Chemical (Ann Arbor, MI). Assays were performed according to manufacturer's instructions.

2.4. Western blot analysis and antibodies

Antibodies for caspase-3, caspase-9, phospho-p38, phospho-JNK, phospho-ASK1 and ASK1, and β-actin were purchased from Cell Signaling Technology (Danvers, MA). Antibodies for JNK, iNOS, and COX-2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The protein content of liver homogenates was measured using a bicinchoninic acid kit (Thermo Scientific, Rockford, IL). Protein aliquots (10–40 μg) were electrophoresed on 4%–20% gradient sodium dodecyl sulfate olyacrylamide gel electrophoresis gels (Life Technologies) and then transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA). The blocked membranes were incubated with primary antibody in Tris-buffered saline Tween20 containing 5% nonfat milk or bovine serum albumin at 4°C overnight, followed by incubation with the appropriate secondary antibodies (Cell Signaling Technology) according to the manufacturer's instructions. Membranes were developed with super-signal west femto maximum sensitivity substrate (Thermo Scientific). Protein loading was routinely confirmed with an antibody against β-actin. Densitometric analysis was conducted using Image Lab Software (Bio-Rad, Hercules, CA).