The therapeutic effect of CORM-3 on acute liver failure induced by lipopolysaccharide/D-galactosamine in mice

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BACKGROUND: Acute liver failure (ALF) is a severe and life-threatening clinical syndrome resulting in a high mortality and extremely poor prognosis. Recently, a water-soluble CO-releasing molecule (CORM-3) has been shown to have anti-inflammatory effect. The present study was to investigate the effect of CORM-3 on ALF and elucidate its underlying mechanism.

METHODS: ALF was induced by a combination of LPS/D-GalN in mice which were treated with CORM-3 or inactive CORM-3 (iCORM-3). The efficacy of CORM-3 was evaluated based on survival, liver histopathology, serum aminotransferase activities (ALT and AST) and total bilirubin (TBiL). Serum levels of inflammatory cytokines (TNF-α, IL-6, IL-1β and IL-10) and liver immunohistochemistry of NF-κB-p65 were determined; the expression of inflammatory mediators such as iNOS, COX-2 and TLR4 was measured using Western blotting.

RESULTS: The pretreatment with CORM-3 significantly improved the liver histology and the survival rate of mice compared with the controls; CORM-3 also decreased the levels of ALT, AST and TBiL. Furthermore, CORM-3 significantly inhibited the increased concentration of pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) and increased the anti-inflammatory cytokine (IL-10) productions in ALF mice. Moreover, CORM-3 significantly reduced the increased expression of iNOS and TLR4 in liver tissues and inhibited the nuclear expression of NF-κB-p65. CORM-3 had no effect on the increased expression of COX-2 in the ALF mice. An iCORM-3 failed to prevent acute liver damage induced by LPS/D-GalN.

CONCLUSION: These findings provided evidence that CORM-3 may offer a novel alternative approach for the management of ALF through anti-inflammatory functions.

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KEY WORDS: acute liver failure; CO-releasing molecule-3; cytokines; inflammation

Introduction

Acute liver failure (ALF) is defined as severe liver injury characterized by increased levels of liver enzymes, hepatic encephalopathy, severe coagulopathy and jaundice.[1, 2] ALF results in extremely high mortality and poor prognosis, despite significant advances in liver transplantation and liver support systems.[3, 4] Although the treatment strategies for ALF have been extensively studied in recent years, there are still no validated therapeutic approaches.[5, 6] Therefore, the study of effective therapies for ALF is of great importance.

Lipopolysaccharide (LPS) and D-galactosamine (D-GalN)-induced acute hepatic damage has been extensively used as an experimental animal model that imitates the pathological processes of human ALF, which is characterized by widespread and massive necrosis of the liver.[7, 8] Increasing evidences indicate that inflammatory responses play a vital role in the pathogenesis of ALF.[9] The release of hepatic and circulating inflammatory cytokines, such as tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-10 (IL-10), is associated with the development and

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prognosis of ALF.[10, 11] Additionally, LPS can stimulate the production of inflammatory mediators such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), which participate in the inflammatory response through the NF-κB signaling pathway.[12] Among the Toll-like receptors (TLRs) family, TLR4 is involved in the pathogenesis of ALF.[13] The inhibition of TLR4 overexpression is also regarded as a potential therapeutic target in LPS/D-GalN-induced ALF.[14] Theoretically, the regulation of inflammatory mediators is considered to be a feasible strategy for the management of LPS/D-GalN-induced ALF.[15]

As an important signaling gas molecule, carbon monoxide (CO) was found to have potent cytoprotective effects including anti-inflammatory, anti-apoptotic, antiproliferative and vasodilator effects.[16-18] However, the therapeutic use of gaseous CO has been limited due to its toxic properties, which result in an increased concentration of carboxyhemoglobin (COHb). CO-releasing molecules (CORMs) have recently been synthesized, and they facilitate the delivery of CO to biological systems without increase of COHb levels.[19] Therefore, CORMs are regarded as very valuable tools to assess CO bioactivity and give rise to new drug candidates for the experimental use of gaseous CO. Among the different classes of CORMs, a water-soluble CORM-3 has been characterized and successfully tested in various animal models of inflammation.[20, 21]

However, it is unclear whether CORM-3 is effective in the treatment of ALF. The present study was to investigate the therapeutic effect of CORM-3 on ALF and its possible mechanisms.

Methods

Animals

Pathogen free C57BL/6 male mice (8 to 10 weeks old) were obtained from the Experimental Animal Center of Harbin Medical University. The mice were caged in a room with standard conditions including light (12-hour light/dark cycle), temperature (22±2 ℃) and humidity (55%±5%). All animals had free access to a standard laboratory diet and water. The mice received humane care in accordance with the guidelines of the Institutional Research Board of Harbin Medical University for the use of experimental animals (HMUIRB20150003).

Reagents

LPS (*Escherichia coli* 011:B4), D-GalN and CORM-3 were purchased from Sigma-Aldrich Chemical Co. LLC (St. Louis, MO, USA); mouse ELISA kits (TNF-α, IL-6, IL-1β and IL-10) were from Blue Gene (Shanghai, China); alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBiL) detection kits were from Siemens Healthcare Diagnostic, Inc. (Newark, USA); rabbit anti-mouse polyclonal antibodies against COX-2, iNOS, TLR4, NF-κB-p65 and β-actin were from Abcam, Inc. (Piscataway, USA); MaxVision™ HRP-Polymer anti-mouse IHC kit (KIT-5030) and MAX007™ (DAB) were from Maixin Biotech, Co., Ltd. (Fuzhou, China).

Experimental groups

Mice were simultaneously injected intraperitoneally with LPS (100 μg/kg) and D-GalN (800 mg/kg) dissolved in phosphate-buffered saline (PBS). The animals were randomly divided into four groups with ten mice in each group: (1) Control group: mice were injected with the same volume of sterile saline alone; (2) LPS/D-GalN group: mice were given only LPS/D-GalN; (3) iCORM-3+LPS/D-GalN group: mice were intraperitoneally administered iCORM-3 (10 mg/kg) dissolved in sterile PBS 30 minutes prior to LPS/D-GalN injection; (4) CORM-3+LPS/D-GalN group: mice were intraperitoneally administered CORM-3 (10 mg/kg) as described above. CORM-3 was dissolved fresh in distilled water on the day of experiment, and was stored at -20 ℃ prior to use. iCORM-3 (inactivated CORM-3) was made by incubating fresh CORM-3 dissolved in PBS and leaving it for 24 hours at room temperature in bubbling with nitrogen to displace the CO.[22] The mice were sacrificed by decapitation at 6 hours after LPS/D-GalN administration. Blood and liver samples were then quickly collected and frozen at -80 ℃ for biochemical and histological analyses. The survival of the mice was assessed using an additional sixty mice grouped as described above 48 hours after LPS/D-GalN administration.

Measurement of serum aminotransferase activities and TBiL

Collected blood samples were centrifuged at 1000×g for 15 minutes to obtain serum samples, which were hemolysis-free and stored at -80 ℃ before use. The serum levels of ALT, AST and TBiL were measured using an automatic chemistry analyser (Dimension®EXL™ with LM, SIEMENS).

Histology

The collected liver tissues were rinsed gently with PBS and preserved in 10% paraformaldehyde. The samples were then dehydrated and embedded in paraffin. The samples were sectioned (5 μm thick) and stained with hematoxylin and eosin (HE) and analysed under a Bio Imaging Navigator microscope (Olympus FSX100, Tokyo, Japan). Histological changes including necro-