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Dopamine alleviated acute liver injury induced by lipopolysaccharide/D-galactosamine in mice

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ABSTRACT

Dopamine (DA), a crucial neurotransmitter, not only functions in the central nervous system but also plays important roles in the modulation of inflammation. Several studies suggest that DA might suppress the inflammatory response both in vitro and in vivo. In the present study, the potential effects of DA in a mouse model with lipopolysaccharide (LPS)/D-galactosamine (D-Gal)-induced acute liver injury were investigated. The results show that DA-treated LPS/D-Gal-exposed mice had reduced incidence of histologic lesions, lower plasma aminotransferases and improved the survival rates compared to LPS/D-Gal-exposed mice. Treatment with DA also suppressed LPS/D-Gal-induced production of TNF-α, phosphorylation of c-jun-N-terminal kinase (JNK), cleavage of caspase-3, up-regulation of hepatic caspase-3, caspase-8, and caspase-9 activities and reduced the count of TUNEL-positive hepatocytes. These data indicate that DA attenuated LPS/D-Gal-induced fulminant liver injury in mice, which implies that DA might have value for the prevention of inflammatory liver disease.

1. Introduction

Acute hepatitis is inflammation/immunity-based liver damage leading to hepatic dysfunction and even death [1]. Acute hepatitis may occur from various origins, including infections, autoimmune factors, drugs, toxins, etc. [1–3]. Lipopolysaccharide (LPS) is a typical toxic component from Gram-negative bacteria [4], which stimulates strong inflammatory responses [5, 6]. D-Galactosamine (D-Gal) is selectively metabolized in hepatocyte and it significantly enhances the sensitivity of the liver to LPS [7]. Hepatic injury induced by LPS/D-Gal in mice closely resembles acute hepatitis of humans [8, 9], and is widely used to investigate the mechanisms of hepatitis and to discover new liver-protective reagents [8, 10].

Dopamine (DA), a pivotal neurotransmitter in the central nervous system, plays crucial roles in the maintenance of emotion, cognition and behavior [11]. In addition, there is growing evidence suggesting that DA is involved in the regulation of immune/inflammatory response [12–15]. It has been reported that DA suppressed the production of tumor necrosis factor alpha (TNF-α) and nitric oxide (NO) in LPS-activated macrophages or glial cells [14, 16]. There have also been studies indicating that DA inhibited the expression of pro-inflammatory cytokines, chemokines and adhesion molecules in neutrophils, endothelial cells or mast cells [17, 18]. These data suggest that DA might be a negative modulator of inflammatory response.

In recent animal studies, treatment with DA suppressed inflammatory response and attenuated tissue injury in mice with acute lung injury and acute pancreatitis [12, 19, 20], suggesting that DA might have value in the intervention of inflammatory injury. Therefore, we questioned whether DA could also provide beneficial effects on the development of acute hepatitis. In the present study, DA was administered in mice with LPS/D-Gal-induced lethal hepatitis, and the survival of the experimental animals, the degree of liver injury and the activation of the pro-apoptotic cascade were determined.

2. Materials and methods

2.1. Reagents

LPS (from *Escherichia coli*, 055:B5), D-Gal and DA were products of Sigma (St. Louis, MO, USA). The alanine aminotransferase (ALT) and

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aspartate aminotransferase (AST) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The enzyme-linked immunosorbent assay (ELISA) kit for detecting mouse TNF-α was produced by NeoBioscience Technology Company (Shenzhen, China). The total protein extract kit and caspase-3, -8, -9 colorimetric assay kits were purchased from Beyotime Institute of Biotechnology (Jiangsu, China). The In Situ Cell Death Detection Kit was obtained from Roche (Indianapolis, USA). The rabbit anti-mouse c-jun-N-terminal kinase (JNK), phosphorylated JNK (p-JNK), cleaved caspase-3 and β-actin antibodies were the products of Cell Signaling Technology (Danvers, MA, United States). The BCA protein assay kit, horseradish peroxidase-conjugated goat anti-rabbit antibody and enhanced chemiluminescence (ECL) reagents were purchased from Pierce Biotechnology (Rockford, IL, USA).

2.2. Animals

Male BALB/c mice (18–20 g), with an average age of 6–8 weeks were purchased from the Experimental Animal Center of Chongqing Medical University (Chongqing, China). The animals were housed in a specific pathogen-free environment in the standard animal room (room temperature: 20–25 °C, humidity: 50 ± 5%) on a regular 12 h:12 h light/dark cycles and fed standard laboratory food and tap water ad libitum. All experiments were conducted in accordance with approved protocols by the University of Chongqing Medical Animal Care and Use Committee.

2.3. Experimental protocol

Acute liver injury was induced in mice by intraperitoneal injection of LPS (10 μg/kg) combined with D-Gal (700 mg/kg). In the present study, 144 mice were allocated into three sets. The first set of animals (32 mice) was randomly divided into 4 groups (n = 8 per group). Group (A) was the control group, and mice received the vehicle only. Group (B) was the DA group, and mice received DA (200 mg/kg, dissolved in frozen phosphate buffer saline). Group (C) was the LPS/D-Gal group, comprised of mice with LPS/D-Gal-induced acute liver injury. Group (D) was the DA + LPS/D-Gal group, and mice received DA (200 mg/kg i.p.) 0.5 h prior to LPS/D-Gal injection. Then, all animals were returned to their cages with food and water available ad libitum. The mice were anesthetized and sacrificed at 1.5 h after LPS/D-Gal challenging, and plasma samples were collected for measuring the level of TNF-α.

The second set of animals underwent the same experimental protocol for evaluating the degree of liver damage (32 mice, divided into 4 groups, n = 8 per group). The animals were sacrificed at 6 h after LPS/D-Gal injection. Blood samples were collected and the livers were harvested.

The third set of animals were allocated (80 mice, divided into 4 groups, n = 20 per group) to determine the mortality rate. The survival of the mice was monitored every 6 h for at least 7 days and the cumulative survival curve was depicted using the Kaplan-Meier method.

2.4. Histological analysis

The right liver lobe of mice were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were stained with hematoxylin & eosin (H&E), then evaluated for the presence of inflammation. The analyses were conducted using a light microscope (Olympus, Japan).

2.5. Determination of liver enzymes

The ALT and AST activities were determined following the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute). Briefly, blood plasma samples were added into the 96-well plate. The ALT or AST matrix fluid was added, and the plate was incubated at 37 °C for 30 min. Then, 2–4-dinitrophenylhydrazine was added into each well, and the plate was incubated at 37 °C for 20 min. Finally, NaOH solution was added, and the levels of ALT or AST were calculated according to the absorbance measured at 490 nm, based on the standard curve.

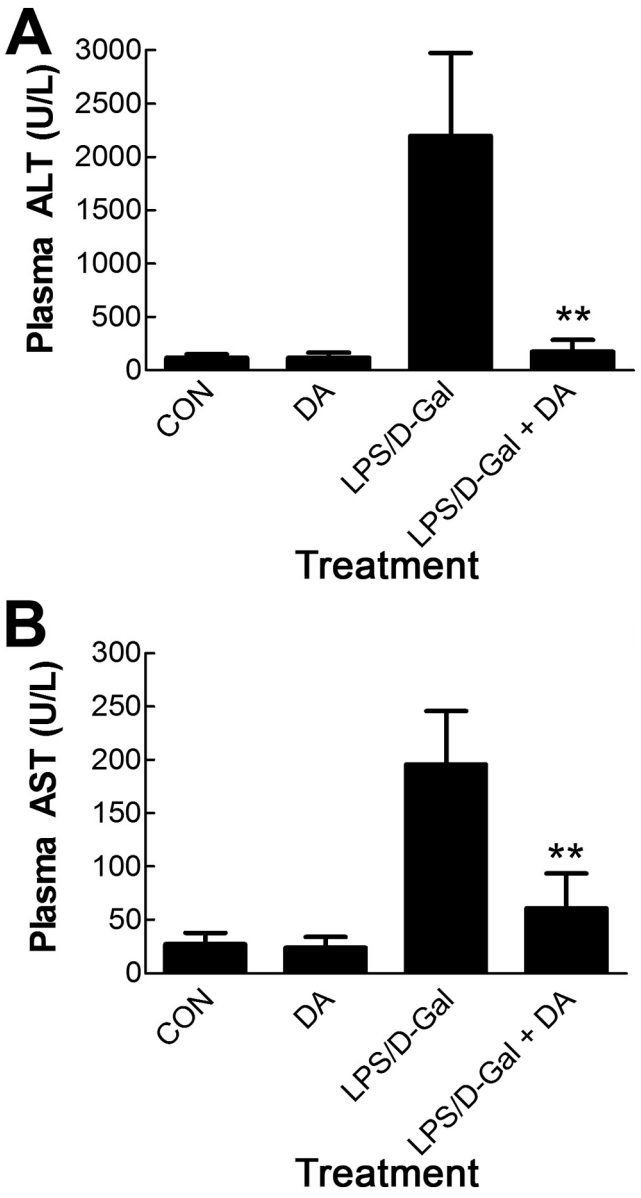

Fig. 1. DA restrained LPS/D-Gal-induced elevation of plasma aminotransferases. Acute liver injury was induced by LPS/D-Gal, and 32 mice were divided into 4 groups (n = 8 per group), DA was injected 0.5 h prior to LPS/D-Gal exposure. Plasma samples were collected at 6 h after LPS/D-Gal exposure. The levels of (A) alanine aminotransferase (ALT) and (B) aspartate aminotransferase (AST) in plasma were determined. Data were expressed as the means ± SD. $^{**}P < 0.01$ compared with the LPS/D-Gal group.

2.6. TNF-α determination by ELISA

The level of TNF-α in plasma was determined using ELISA kits (NeoBioscience) following the manufacturer's protocols. Briefly, all reagents were brought to room temperature (RT) prior to use. The plasma samples were added into the 96-well plate containing specific enzymes and the plate was incubated at 36 °C for 90 min. The plate was washed 5 times with the wash buffer and firmly tapped on absorbent paper. Then the detection antibody solution was added into each well, and the plate