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Caloric restriction mimetic 2-deoxyglucose alleviated lethal liver injury induced by lipopolysaccharide/D-galactosamine in mice

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ABSTRACT

The glycolytic inhibitor 2-deoxyglucose (2-DG) is a calorie restriction (CR) mimetic produces CR-like beneficial effects in both acute and chronic pathological processes, but whether 2-DG is also helpful in critical and life-threatening situation is not known. In the present study, the potential benefits of 2-DG in lipopolysaccharide/D-galactosamine (LPS/D-Gal)-induced lethal liver injury were investigated. The results indicated that treatment with 2-DG suppressed the elevation of plasma aminotransferases, alleviated the histopathological abnormalities and improved the survival rate of LPS/D-Gal-exposed mice. Treatment with 2-DG also suppressed the production of pro-apoptotic cytokine TNF-α, the phosphorylation of JNK, the activation of caspase cascade and the count of TUNEL-positive apoptotic hepatocytes. These data suggested that the CR mimetic 2-DG could also provide beneficial effects in lethal pathological process such as LPS/D-Gal-induced fulminant liver injury.

1. Introduction

Calorie restriction (CR), which refers to restricting the intake of calories, has emerged as an active research area because it is a reliable intervention can effectively prolong lifespan and increase healthspan [1]. However, long-term CR would be highly problematic because of compliance challenge and other unpleasant side effects [2]. Therefore, the concept of a CR mimetic has been proposed. CR mimetic can mimic metabolic, hormonal, physiological effects of CR and produces CR-like beneficial effects on longevity and health without significantly reducing food intake [3]. The identification of CR mimetic provides novel promising approaches for the maintenance of health [2].

The glycolytic inhibitor 2-deoxyglucose (2-DG) was proposed as the first CR mimetic [4]. 2-DG blocks glycolysis primarily by competitively inhibiting phosphoglucose isomerase (PGI) because 2-DG is phosphorylated by hexokinase (HK) to form 2-DG-6-phosphate (2-DG-6-P) and 2-DG-6-P can compete with the fructose-6-phosphate (F-6-P) for PGI [5]. There is accumulative evidence indicating that treatment with 2-DG would be beneficial in chronic pathological processes such as cancer, Alzheimer's disease and Parkinson's disease [6–8]. In addition, several studies also found that administration of 2-DG protected against acute ischemia injury in rat [9,10]. Therefore, the CR mimetic 2-DG might also be helpful in acute pathological processes.

To further expand the investigation of CR mimetic, we questioned whether 2-DG could also provide beneficial effects in more serious and life-threatening situation. Recent studies have found that 2-DG suppressed lipopolysaccharid (LPS)-induced cytokine production in monocytes and inhibited the phagocytosis capacity of LPS-stimulated macrophages [11,12]. LPS, the major virulence factor of Gram-negative bacteria, is one of the representative pathogenic causers of lethal tissue injury [13,14]. Administration of LPS in D-galactosamine (D-Gal)-sensitized mice could selectively induce lethal liver injury that closely resembles clinical hepatitis in human [15]. In the present study, the potential effects of 2-DG on the degree of liver injury and the mortality of mice with LPS/D-Gal-induced lethal liver injury was investigated.

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2. Materials and methods

2.1. Materials

2-DG, LPS (from *Escherichia coli*, 055:B5) and D-Gal were purchased from Sigma (St. Louis, MO, USA). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) assay kits were produced by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) kit for detecting mouse TNF-α was the products of 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). In Situ Cell Death Detection Kit was purchased 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 purchased from 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 obtained from Pierce Biotechnology (Rockford, IL, USA).

2.2. Animals

Male BALB/c mice weighing 20–25 g were obtained from the Experimental Animal Center of Chongqing Medical University. The animals were housed in a specific pathogen-free room at a temperature of 20–25 °C and 50 ± 5% relative humidity under a 12-h dark/light cycle. All animals were fed with a standard laboratory diet and water ad libitum. All experimental procedures involving animals were approved by the Animal Care and Use Committee of Chongqing Medical University.

2.3. LPS/D-Gal-induced liver injury

LPS (10 μg/kg) and D-Gal (700 mg/kg) were injected intraperitoneally in mice to induce lethal hepatitis. The vehicle or a serial dose of 2-DG (125 mg/kg, 250 mg/kg, 500 mg/kg and 1000 mg/kg, dissolved in NS, i.p.) was administrated 0.5 h prior to LPS/D-Gal injection. Then, the animals were returned to their cages and allowed food and water ad libitum. To determine the degree of liver damage, mice were sacrificed at 6 h after LPS/D-Gal injection. The liver and plasma samples were harvested for morphological examination, aminotransferases determination and other biochemical analyses. To determine the degree of inflammation, another set of animals were sacrificed at 1.5 h after LPS/D-Gal injection. The plasma and liver samples were harvested for measuring the level of TNF-α. To determine the mortality, survival of the third set of mice (n = 20 per group) was assessed four times a day for at least 7 days and the cumulative survival curve was depicted using the Kaplan–Meier method.

2.4. Histological analysis

The liver tissues were fixed in formalin, embedded in paraffin and stained with hematoxylin & eosin for histopathological evaluation under light microscope (Olympus, Japan).

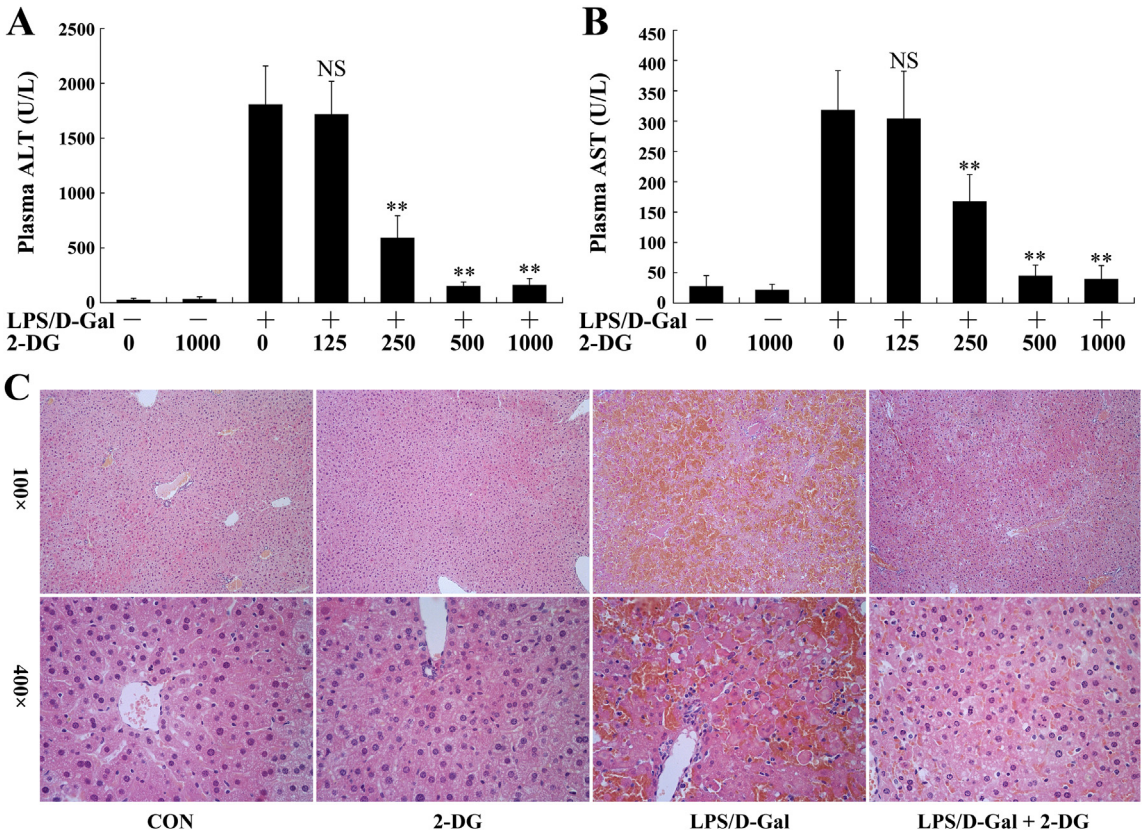

Fig. 1. 2-DG suppressed LPS/D-Gal-induced liver damage. Mice were treated with vehicle or various doses of 2-DG (125 mg/kg, 2500 mg/kg, 500 mg/kg and 1000 mg/kg) in the absence or presence of LPS/D-Gal challenge. The levels of (A) alanine aminotransferase (ALT) and (B) aspartate aminotransferase (AST) in plasma were determined at 6 h after LPS/D-Gal exposure. Data were expressed as mean ± SD, n = 8. NS $P > 0.05$, $^{**}P < 0.01$, compared with the LPS/D-Gal group (LPS/D-Gal +/2-DG 0). (C) Mice were treated with vehicle or 2-DG (500 mg/kg) in the absence or presence of LPS/D-Gal challenge. Liver samples were harvested at 6 h after LPS/D-Gal exposure and the liver sections were stained with hematoxylin-eosin for morphological evaluation. The representative liver sections of each group are shown (original magnification 100 and 400).