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The protective effect of juglanin on fructose-induced hepatitis by inhibiting inflammation and apoptosis through TLR4 and JAK2/STAT3 signaling pathways in fructose-fed rats

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ABSTRACT

High fructose-feeding is an essential causative factor leading to the development and progression of hepatitis associated with high levels of endotoxin (LPS). Juglanin, as a natural compound extracted from the crude Polygonum aviculare, displayed inhibitory activity against inflammation response and cancer growth. However, researches about its role on anti-inflammation and apoptosis are far from available. Here, it is the first time that juglanin was administrated to investigate whether it inhibits fructose-feeding-induced hepatitis in rats and to elucidate the possible mechanism by which juglanin might recover it. Fructose-feeding rats were orally administrated with juglanin of 5, 10 and 20 mg/kg for 6 weeks, respectively. Juglanin exerted prevention of fructose-feeding-stimulated increased LPS levels, accelerated alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) and up-regulated inflammatory cytokines expression in serum, mainly including tumor necrosis factor-alpha (TNF-a), Interleukin 1beta (IL-1β), Interleukin 6 (IL-6) and Interleukin 18 (IL-18). Meanwhile, toll-like receptor 4 (TLR4)-modulated mitogen-activated protein kinase (MAPK)/nuclear factor kappa B (NF-κB) and apoptosis-related Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling pathway are involved in the progression of hepatic injury and inflammation. And juglanin was found to suppress fructose-feeding-induced activation of these signaling pathways compared with the model group administrated only with fructose. These results indicate that juglanin represses inflammatory response and apoptosis via TLR4-regulated MAPK/NF-κB and JAK2/STAT3 signaling pathway respectively in rats with hepatitis induced by LPS for fructose-feeding. Treatment of juglanin might be an effective therapeutic strategy for preventing hepatitis.

1. Introduction

Hepatitis is a medical condition defined by the inflammation of the liver and characterized by the presence of inflammatory cells in the tissue of the organ [1]. Hepatitis is acute when it lasts less than six months and chronic when it persists longer. Acute hepatitis can be self-limiting, can progress to chronic hepatitis, or, rarely, can cause acute liver failure, and may progress over time to fibrosis and cirrhosis [2]. Cirrhosis of the liver increases the risk of developing hepatocellular carcinoma, known as a form of liver cancer [3]. Worldwide, viral hepatitis is the most common cause of liver inflammation [4]. Other causes include autoimmune diseases and ingestion of toxic substances, and nonalcoholic fatty liver disease. Thus, studies to find effective strategies for ameliorating hepatitis are necessary presently. Fructose has been found to induce nonalcoholic fatty liver disease (NAFLD), the third most common cause of liver disease in the United States [5,6]. Thus here, fructose was administrated for rats to induce hepatitis.

Interestingly, the persistent replication of inflammation response is closely associated with the progression and development of infection as well as the occurrence of Toll like receptor 4 (TLR4)

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Table 1
Primer sequences of RT-PCR analysis.

Gene	Forward primers (5′-3′)	Reverse primers (5′-3′)
GAPDH	CATTCAAGACCGGACAGAGG	ACATACTCAGCACCAGCATCACC
IFN-γ	GCAAAGGGAACATTCCGATAT	GCGACATCACATGGAAATCTA
IL-17	GAACCGGCACCTGACACC	ACGACCTTCGTCAGTAC CGA
IL-10	AGAACACAGAGTCAGTGGC	CCAGTGAGTTATGTCTGG
IL-1β	GACAGCAAGATAGTAGGCC	CGGAAGCTTCTTGTATGTGGG
IL-18	GCACAGTGTGAGAGGGCGGT	CTGTACCTGCGTCGTGCTCTGT
IL-6	CATGTGGGCCGTGCACAG	TTCCGTGACCCTTTGGAACT
IL-35	CAGAAGGAAGTTAGGCC	CGTCGCAGTGGATGATGTG
TNF-α	CTTCTCACTGTCGACTACCGC	GCGTC TCCTGTGCATTCG
TGF-β	GACTCTTCCTGGTCTTACCATATT	CTGCTATTGCAAGGACCCAATT
JAK2	CAGAACAGGAGGGACCGCCA	CCACGCCATTTCCTCCCTCAT
STAT3	CAAGTCTGTCTCACTGCAGATAGTG	CTGCCCTATTACCCTTGTTTCG
Bcl-Xl	CAGACAAGAAGAGGTTGCC	CGTCAGTCAGTGTGTATG
Cyto-c	CAGACAAGAAGAGGTTGCC	CGTCATGGCAGTGTGTATTGG
Caspase-9	CAGACAAGAAGAGGTTGCC	CGTCAGGCAGTTTGTATTGG
Caspase-3	CAGACAAGAAGAGGTTGCC	CGTCAGGCAGTTTGTATTGG

regulated inflammation [7]. Previous study indicated the crucial role of TLR4 in LPS-induced inflammation [8]. The receptor of LPS, as a remarkable regulator of the inflammation resulting in infection and poison, plays an important role in the progression of a variety of human diseases, involving cancer and hepatitis [9,10]. LPS could activate interaction between TLR4 and MyD88/TAK1, and further activate MAPK and NF-kB signaling pathways to stimulate inflammation [11]. Therefore, how to inhibit TLR4-regulated inflammatory signaling pathway effectively might be a key strategy for drug screening to reverse LPS-induced inflammatory response. Although with the advance of knowledge for therapy and diagnosis, the effective treatment method and drugs are still far from to be understood.

Recently, juglanin, as a natural compound extracted from the crude Polygonum aviculare, displayed inhibitory activity against inflammation response and cancer growth [12]. The inhibitory activities of juglanin against LPS-induced cytokine, such as TNF-α, IL-1β, and IL-6 production in macrophage cells have been evaluated [13]. Study showed that juglanin reduced LPS-induced expression of iNOS and COX-2 proteins significantly. However, there are no related reports about the anti-inflammatory and apoptotic effects of juglanin and the underlying mechanism [14,15]. Thus, we used fructose-feeding rats to investigate the anti-inflammatory effect of juglanin on hepatitis and nerve injury.

2. Methods and materials

2.1. Animals

A total of 75 male Sprague-Dawley rats ranging from 8 to 10 weeks old, 200 ± 20 g weight, were purchased from the Experimental Animal Centre. They were maintained in a controlled environment individually with 12:12 h light/dark cycle at a temperature of 22 ± 2 °C and a relative humidity of 55%. Water and food were provided *ad libitum*. Animal welfare and experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (The Ministry of Science and Technology of the People's Republic of China, 2006). Rats were randomly divided into five groups: control group with conventional diet (Con), fructose-feeding group (Veh) with 10% (wt/vol) fructose in drinking water and standard chow for 10 weeks, fructose-feeding diet with juglanin of 5 mg/kg (L), 10 mg/kg (M) and 20 mg/kg (H). Rats were administrated with different concentration of juglanin orally for 5 weeks.

2.2. Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

After 5-weeks drug treatment, an oral glucose tolerance test (OGTT) was performed following a 16-h fasting. One hour after drug administration, the rats were administered orally with glucose (1.5 g/kg) as a 50% glucose solution. Tail-vein blood samples were collected at 0, 30, 60, 90 and 120 min after glucose treatment. For ITT, insulin 0.3 U/kg dissolved in 1 ml 0.9% NaCl was injected in fed rats. Blood samples were taken from the tail vein at 0, 30, 60, 90 and 120 min after injection. The samples were centrifuged (3000*g*) at 4 °C for 10 min and serum samples were separated.

2.3. Sample collection

After OGTT and ITT, all animals were allowed 3 days to recover wounds and then killed by decapitation at 9:00–10:00 a.m. after a 16-h fasting. Liver samples were rapidly dissected quickly on ice, weighed, and parts of them were immediately fixed for oil-red O and hematoxylin–eosin staining, respectively, while others were stored in liquid nitrogen for Western blot analysis. Blood samples were centrifuged (3000*g*, 4 °C) for 10 min to get serum stored at −80 °C for biochemical assays.

2.4. ELISA analysis

Interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-18 (IL-18) et al. levels in serum and liver were determined by ELISA kits following the manufacturer's instructions strictly.

2.5. Biochemical analysis

For rat hepatic function examination, serum and levels of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were determined using common biochemical kits. Rat liver tissues were weighed and extracted. The chloroform layer and serum samples were used to determine TC, TG, high density lipoprotein (HDL) and low density lipoprotein (LDL) levels by use of common commercially-available biochemical kits.

2.6. H&E staining and flow cytometry analysis

The collection of liver samples was subjected to hematoxylin-eosin (H&E) staining and was examined for liver injury by light microscopy. For flow cytometry analysis, the lymphocytes were obtained through shearing liver tissue, separating by collagenase type II from Invitrogen in USA digestion and suspended in RPMI 1640 medium (GIBCO, BRL). Cell suspensions were centrifuged at 1000 rpm for 5 min to remove cellular debris and impurities. Then, the hepatic mononuclear cells (MNCs) were harvested and re-suspended in 70% percoll (Sigma). MNCs were collected from the interphase, and washed twice in Hank's buffer (GIBCO, BRL). According to the protocol of R&D kit systems (R&D, USA) for flow cytometry, add anti-TLR4 FITC antibody to the flow cytometry tube containing single-cell suspension, and these tubes were analyzed by Cytomics™ FC 500 MCL of Beckman coulter (BECKMAN, USA).

2.7. Immunoinfluorescence assays

Immunoinfluorescent assay of TLR4 and *p*-NF-κB (Abcam, Shanghai, China) for liver samples were performed according to the instructions of the manufacturer.