International Immunopharmacology 56 (2018) 105–112

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Oxyresveratrol prevents lipopolysaccharide/D-galactosamine-induced acute liver injury in mice

Ya-Nan Jia[a], Hai-Peng Lu[a], Ya-Lin Peng[a], Bao-Shun Zhang[b], Xiao-Bao Gong[b], Jun Su[c], You Zhou[a], Min-Hui Pan[a], Li Xu[a,*]

[a] College of Biotechnology, Southwest University, No. 2 Tiansheng Road, BeiBei District, Chongqing 400715, China
[b] College of Pharmaceutical Sciences, Southwest University, No. 2 Tiansheng Road, BeiBei District, Chongqing 400715, China
[c] Guangan Vocational Technical College, No. 98 Binjiang East Road, Guangan, Sichuan 638550, China

ARTICLE INFO

Keywords:
Oxyresveratrol
Acute liver injury
Lipopolysaccharide/D-galactosamine
TLR4/NF-κB
Keap1-Nrf2
Anti-apoptosis

ABSTRACT

Oxyresveratrol (Oxy) is a natural polyhydroxystilbene abundant in mulberry that has anti-inflammation and anti-oxidant activities. We evaluated the protective effect of Oxy in the context of the lipopolysaccharide and D-galactosamine (LPS/D-GalN) induced acute liver injury. Oxy restricted the development of histopathological changes, markedly reduced the activity of alanine transaminase (ALT) and aspartate transaminase (AST), which are indicators of impaired liver function. Oxy significantly regulated the contents of oxidative stress related enzymes and products, and inhibited expressions of inflammatory mediators and cytokines. Oxy treatment diminished the Toll-like receptor 4/nuclear factor-kappa B (TLR4/NF-κB) signaling pathway in liver, activated the Kelch-like ECH-associated protein 1(Keap1)-nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, and increased expressions of heme oxygenase 1 (HO-1) and quinine oxidoreductase 1(NQO1). Pretreatment with Oxy decreased LPS/D-GalN stimulated hepatocyte apoptosis by efficaciously raising the B-cell lymphoma 2 (Bcl-2)/Bcl-2 associated X (Bax) ratio, inhibiting the expression and activation of caspases, and activating the phosphoinoside-3-kinase (PI3K)-Akt pathway. Our results demonstrate the hepatoprotective efficacy of Oxy. The protection is mainly due to the prevention of TLR4/NF-κB pathway activation, induced activation of Keap1-Nrf2 signaling pathway, and decreased hepatocyte apoptosis. Oxy warrants further study as a potential therapeutic agent candidate for the management of acute liver injury.

1. Introduction

Acute liver injury (ALI) is a multifactorial complex inflammatory disease [1] resulting from various etiologies, including viral hepatitis and alcohol or drug abuse [2,3]. ALI is associated with high mortality in the clinic. LPS, a major ingredient of the Gram-negative bacterial cell well. The liver toxin, D-GalN potentiates the toxic effects of LPS and can trigger fulminant hepatitis, which can lead to clinical ALI within few hours in rats [4,5,6].

When hepatocytes are attacked by LPS, TLR4, which recognizes LPS, with the assistance of myeloid differentiating factor 88 (MyD88), activates the nuclear transcription factor NF-κB [7,8], which regulates the expression of genes, including interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) [9]. The TLR4/NF-κB pathway is considered one of the main signaling pathways involved in the acute inflammatory response.

Inflammation is often accompanied by formation of reactive oxygen species (ROS) and oxidative stress [10]. The Keap1-Nrf2 pathway regulates an adaptive cellular defense response to inflammation and oxidative stress [11,12]. During homeostasis, Nrf2-dependent transcription is repressed by a negative regulator, Keap1. But when the cells face oxidative stress, the Keap1-Nrf2 signaling pathway is activated. By changing the binding state, Nrf2 is released from Keap1 and translocate into the nucleus where it binds to the anti-oxidant responsive elements (AREs) located in the promoter of many cytoprotective genes. The result is the initiation of the synthesis of various anti-oxidant enzymes like HO-1 and NQO1, thereby exerting its anti-inflammatory, anti-oxidative and anti-apoptotic effects [13,14,15].

Apoptosis is the main pathological feature of LPS/D-GalN-induced ALI [16,17]. In the liver tissue, LPS activates the NF-κB signaling pathway and increases the expression of inflammatory cytokines. Caspases are the executioners of apoptosis. They are activated by inflammatory cytokines and cleavage of specific proteins, resulting in irreversible hepatocyte cell death and induced liver failure [18]. PI3K-

* Corresponding author.
E-mail address: mulberry@swu.edu.cn (L. Xu).

https://doi.org/10.1016/j.intimp.2018.01.014
Received 8 November 2017; Received in revised form 7 January 2018; Accepted 9 January 2018

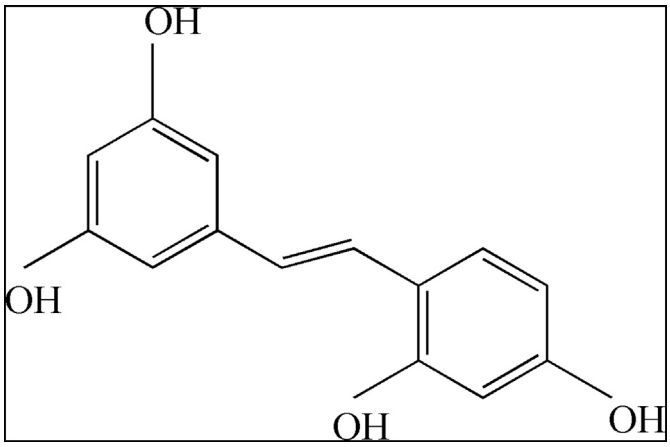

Fig. 1. The structure of oxyresveratrol.

Akt signaling pathway is important for the regulation of cell survival and apoptosis [19,20]. At least in part, in the PI3K-Akt pathway, phosphorylate Akt mediates the inactivation of caspase 9, which suppresses apoptosis and promotes cell survival [21,22].

Oxyresveratrol (Oxy, trans-2′,3,4′,5-tetramethoxystilbene, Fig. 1), is a polyphenolic phytoalexin produced by *Morus alba* L. [23]. Oxy is a powerful free radical scavenger and tyrosinase inhibitor, which has various bioactivities, including anti-inflammatory activity [24], abrogates oxidative stress [25], anti-viral [26], and protects cells [27]. Oxy may have potential in the treatment of acute liver damage. Thus, determining its mechanism of action is important. Recent studies indicated that the anti-inflammatory properties of Oxy might involve blocking the mitogen-activated protein kinase and NF-κB signaling pathways and inhibiting the activities of inflammation-related enzymes [28,29]. Oxy can protect against liver injury by increasing the translocation of Nrf2 and the expression of anti-oxidative enzymes, decreasing the content of malondialdehyde (MDA) and TNF-α [30,31]. In vitro and vivo studies have shown that Oxy attenuates acute inflammation, abrogates oxidative stress, and inhibits apoptosis [32,33]. Still, the combined evaluation of all these molecular mechanisms on mammalian models has not been done. Therefore, the aim of this study was to gain a deeper understanding of the mechanisms of the protective action of Oxy on LPS/D-GalN-induced ALI. We investigated the protective effect of Oxy associated with the regulation of the TLR4/NF-κB, Keap1-Nrf2, and PI3K-Akt-apoptotic pathways, and the expression of the related enzymes and specific proteins.

2. Materials and methods

2.1. Materials

Oxy (purity 99% by high performance liquid chromatography) was purchased from Sigma-Aldrich (St. Louis, MO, USA). LPS from *Escherichia coli* 055:B5 was obtained from Sigma-Aldrich (Shanghai, China). D-GalN was acquired from Aladdin Reagent Database, Inc. (Shanghai, China). Silymarin (Sily) was obtained from MADAUS AG Co., (Chongqing, China). Diagnostic kits for inducible nitric oxide synthase (iNOS), MDA, catalase (CAT), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), ALT, and AST were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Total protein extraction kit and nucleoprotein extraction kit were obtained from Sangon Biotech Co. Ltd. (Shanghai, China). Rabbit primary polyclonal antibodies to β-actin, TLR4, MyD88, IL-6, inhibitor kappa B (IκB), *p*-IκB, IκB kinases (IKKs), *p*-IKKα/β, NF-κB (p65), Nrf2, Keap1, HO-1, NQO1, PI3K, *p*-PI3K, Akt, *p*-Akt, Bax, Bcl-2, caspase3, caspase8, caspase9, lamin B and mouse IL-1β, and TNF-α were purchased from Proteintech (Wuhan, China). Goat anti-rabbit IgG horseradish peroxidase (HRP)-conjugated secondary antibody and goat anti-mouse IgG-HRP-conjugated secondary antibody bought from Sangon Biotech Co. Ltd. (Shanghai, China).

2.2. Animals and treatment

Male Kunming mice (20 ± 2 g) were purchased from Chongqing Academy of Chinese Materia Medica. Mice were maintained under standard environmental conditions of temperature (25 °C) and humidity (50%), and had free access to sterile food and water. After 6 days of acclimation, the mice were randomly divided into five groups with 10 mice in each group. The control group was fed with normal saline. The LPS/D-GalN group was fed with normal saline for 6 days and 6 h after the final saline administration were injected with LPS (50 μg/kg body weight) and D-GalN (500 mg/kg body weight) dissolved in saline. The Oxy group received Oxy at 40 and 80 mg/kg body weight per day for 6 consecutive days, with LPS/D-GalN injected 6 h following the last Oxy treatment. The positive control group was treated with a continuous administration of Sily, which is the most commonly prescribed drug for hepatitis, (54 mg/kg body weight/day) for 6 days, with LPS/D-GalN injected 6 h after the final Sily administration. Animals were sacrificed for an experimental evaluation under anesthesia within 7 h of administration of LPS/D-GalN.

2.3. Ethics statement

All animal experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals, which published by National Institutes of Health and access to the Southwest University Laboratory Animal Management Committee approved.

2.4. Histopathological examination

Several liver tissues were fixed in 10% formaldehyde solution, embedded in paraffin, cut into 5 μm-thick continuous sections, and stained with hematoxylin and eosin (H&E). The histopathological changes of liver tissues were observed by fluorescence microscopy using a TE2000 microscope (Nikon, Tokyo, Japan). Representative pictures are presented.

2.5. Serum determinations

Following the manufacturer's instructions for the commercial assay kits (Nanjing Jiancheng Biotechnology Institute, China) the serum levels of ALT, AST, CAT, SOD, T-AOC, iNOS, and MDA were determined.

2.6. Western blotting analysis

Total proteins or nuclear proteins were extracted according to the instructions of the commercial assay kits (Sangon Biotech Co. Ltd., Shanghai, China). Total homogenates and cytosolic and nuclear fractions were added with a 5× solution of sample buffer and heated at 95 °C for 10 min. The proteins were separated by 12% SDS-PAGE and transferred to polyvinylidenedifluoride membranes. Blots were blocked in 10% nonfat milk at room temperature for 2 h, incubated with appropriate primary antibodies at 4 °C overnight, and incubated with secondary antibodies for 2 h at room temperature. Proteins were visualized and quantitated using Image Jet software. Total homogenate and cytosolic protein bands were normalized to the β-actin content and nuclear fractions bands were normalized to the Lamin B content.

2.7. Statistical analysis

All data are presented as mean ± S.D. The statistical significance of the differences between groups was determined by ANOVA in SPSS 19.0. Statistical differences were considered significant when $p < .05$,