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Baicalein pretreatment protects against liver ischemia/reperfusion injury via inhibition of NF-κB pathway in mice

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ABSTRACT

Ischemia/reperfusion (I/R) is a pathophysiologic process that occurs during hemorrhagic shock, liver resection and liver transplantation. Baicalein, the main active ingredient of the Scutellaria root, exerts anti-inflammatory and anti-apoptotic properties in the setting of I/R injury in the heart and brain. However, the role of baicalein in liver I/R injury and its regulatory mechanisms remain poorly understood. This study was designed to evaluate the effects of baicalein in a model of liver I/R in mice and to explore the possible mechanisms. Baicalein (100 mg/kg) was intraperitoneally injected 1 h before warm ischemia. Pretreatment with baicalein protected against liver I/R injury, as indicated by the decreased serum aminotransferase levels and the reduced histopathologic abnormalities. Baicalein also significantly reduced cellular hepatic apoptosis in response to I/R injury. Moreover, pretreatment with baicalein significantly inhibited nuclear factor-kappa B (NF-κB) activation and the subsequent proinflammatory cytokine production, and decreased leukocyte infiltration. In vitro studies, baicalein treatment inhibited the proinflammatory cytokine production via the modulation of NF-κB signaling pathway in lipopolysaccharide-stimulated macrophages. Taken together, these results suggest that baicalein could protect against liver I/R injury via inhibition of inflammation by down-regulating NF-κB activity, and suppression of cellular hepatic apoptosis.

1. Introduction

Ischemia/reperfusion (I/R) injury is triggered when the liver is transiently deprived of oxygen and reoxygenated. It is a leading cause of liver injury after liver transplantation, liver resection and trauma [1,2]. I/R injury is a complex phenomenon leading to an injurious inflammatory response, which is characterized by the activation of resident Kupffer cells and the infiltration of leukocytes, as well as the production of inflammatory cytokines [3]. Activated immune cells (e.g., Kupffer cells) release a large amount of inflammatory mediators, including reactive oxygen species (ROS), tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1. These inflammatory mediators result in a direct cellular damage, and indirectly lead to the migration and accumulation of neutrophils in the liver via inducing the expression of chemokines and adhesion molecules [4,5]. Activated neutrophils result in additional, prolonged injury via the release of oxidants and proteases [6].

Baicalein (5,6,7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one) is a main active ingredient derived from the dried root of Scutellaria, which is a popular herb in traditional Chinese medicine. Baicalein shows a variety of biological activities, including anti-inflammatory [7], anti-apoptotic [8], anti-oxidant [9], anti-thrombotic [10], and anti-cancer [11] properties. Previous investigations have shown that baicalein acts as an anti-inflammation agent, inhibits the lipopolysaccharide (LPS)-induced inflammation, improves the vasoreactivity and the survival rate, as well as reverses the organ injury in septic animals [12,13]. Baicalein also exerts a cytoprotective role in H_2O_2-induced apoptosis by inhibiting the mitochondria-dependent caspase activation [8]. Recent studies have demonstrated that baicalein is able to decrease I/R injury in the brain and heart [14,15]. Moreover, baicalein protects animals from D-galactosamine(GalN)/LPS induced acute liver failure [16] and carbon tetrachloride (CCl_4)-induced liver damage [17] via inhibition of inflammation and apoptosis in murine models. However, its impact on liver I/R injury and its molecular mechanisms remain unclear.

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Prompted by the close associations between the pathological features of liver I/R and the protective properties of baicalein, we hypothesized that baicalein could protect against hepatic I/R injury. To test this hypothesis, we investigated the beneficial effects of baicalein, as well as its plausible signaling mechanisms in a mouse model of liver I/R.

2. Materials and methods

2.1. Experimental design

Experimental design was described in Fig. 1. To investigate whether baicalein could protect against liver I/R injury, mice were pretreated with either baicalein (100 mg/kg, i.p. Sigma-Aldrich, St. Louis, MO) or vehicle (dimethyl sulfoxide, DMSO, Sigma-Aldrich) 1 h prior to warm ischemia. Mice were killed at 1, 6 and 24 h of reperfusion. Liver injury, inflammatory cytokine, neutrophil infiltration, apoptosis and nuclear factor-kappa B (NF-κB) activation were analyzed.

To investigate the possible mechanism of the anti-inflammatory effects of baicalein, murine macrophage cell line RAW264.7 and primary murine peritoneal macrophages were used in vitro cell culture studies. RAW264.7 cells and peritoneal macrophages were pretreated with various doses of baicalein (1–10 μm) or vehicle (DMSO) for 1 h and then exposed to LPS (*Escherichia coli* serotype O55:B05 type, Sigma-Aldrich, 10 ng/mL) for different periods. The production of inflammatory cytokine and the activation of NF-κB were analyzed.

2.2. Animals

Male inbred C57BL/6 mice (8–10 weeks old, weighing within 20–22 g) were purchased from Wuhan university Center for Animal Experiment (Wuhan, China). All animals were housed under standard animal care conditions and had free access to water and food. All procedures were carried out according to the ethical guidelines of the Animal Care and Use Committee of Huazhong University of Science and Technology.

2.3. Partial hepatic warm I/R

The partial hepatic warm I/R model was generated as described previously [18]. In brief, mice were completely anesthetized with pentobarbital (60 mg/kg, i.p.). After opening the abdomen and dissecting the interlobular ligaments, a microvascular clamp was used to interrupt the arterial and portal venous blood supply to the left lateral and median liver lobes for 60 min.

2.4. Liver and kidney damage assessment

Serum alanine transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine levels were measured using an automated chemical analyzer (Hitachi Co, Tokyo, Japan).

2.5. Histopathology

Liver tissue was fixed in 4.5% buffered formalin for at least 24 h. Paraffin embedding was performed using standard techniques. Sections (4 μm) were stained with Hematoxylin–Eosin and assessed for tissue damage.

2.6. Myeloperoxidase (MPO) immunohistochemistry

The presence of neutrophils in the liver was assessed by myeloperoxidase (MPO) staining. Briefly, after de-paraffinization, rehydration and antigen retrieval, the sections were incubated with MPO antibody (1:50; Abcam, Cambridge, UK) for 1 h at room temperature. After washing, the sections were incubated with goat anti-rabbit secondary antibody and visualized with diaminobenzidine. MPO-positive neutrophils were counted in 5 high-power fields (HPFs) per section at a magnification of 400×. The results are expressed as the mean number of MPO-positive neutrophils per HPF.

2.7. Caspase-3 activity assay

Relative caspase-3 activity in the liver tissues was detected with a caspase-3 colorimetric assay kit (Abcam) according to the manufacturer's instructions.

2.8. RAW264.7 and peritoneal macrophage cell culture

The murine macrophage cell line RAW264.7 was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Peritoneal macrophages were isolated as described previously [19]. Cells were plated in 24-well plates at a density of 5×10^5 cells/well for cytokine assay or in 6-well plates at a density of 2×10^6 cells/well for Western blotting.

2.9. Assessment of cell viability

RAW264.7 cells were cultured in a 96-well plate at a density of 5×10^3 cells/well. Following cultured with baicalein at different doses

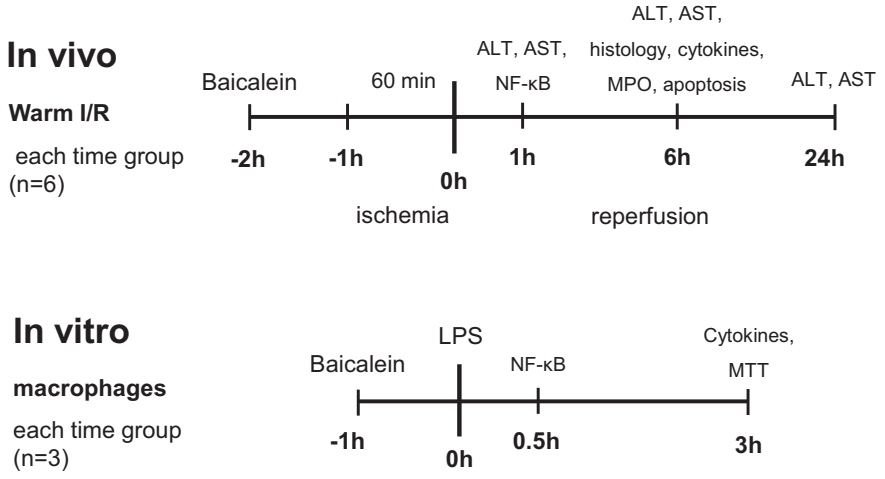

Fig. 1. Schematic diagram of the experimental protocol.