Protective Effects of Chalcone Derivatives for Acute Liver Injury in Mice

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INTRODUCTION

Fulminant hepatic failure is a clinical syndrome resulting from massive death (apoptosis or necrosis) of hepatocytes (Kosai et al., 1999). Fulminant hepatic failure is induced in diverse pathological conditions such as hepatitis viral infection, drug and toxin exposure, alcohol, ischemia, metabolic disorder, massive malignant infiltration, septic shock, and chronic autoimmune hepatitis (Mas et al., 1997). Different therapeutic options used to treat fulminant hepatic failure include antibiotics, diuretics, corticosteroids, blood transfusion, charcoal hemofusion, plasmapheresis and liver transplantation (Kim et al., 2000). However, none of these methods have been shown to effectively treat fulminant hepatic failure. A potent therapeutic agent that can prevent massive hepatocytic cell death is critical for the treatment of fulminant hepatic failure. Tumor necrosis factor-alpha (TNF-alpha)-induced massive hepatocyte apoptosis is a predominant mechanism functioning in this model (Kawaguchi, 1999; Sass et al., 2002).

Chalcone derivatives, one of the large families of plant constituents, have various therapeutic benefits, including antioncogenic (Kumar et al., 2003), anti-inflammatory (Hiseh et al., 1998), analgeisc (Viana et al., 2003), antiulcerative (Murakami et al., 1991), antiviral (Wu et al., 2003), antibacterial (Bekhit et al., 2001), antifungal (Lopez et al., 2001) and antimalarial (Liu et al., 2001) properties. Butein (Fig. 1) is the nature chalcone derivative obtained from Dalbergia odorifera and has antioxidative activity (Cheng et al., 1998). It had been reported by Woo et al. that butein has weak hepatoprotective effects on rat liver injury (Woo et al., 2003). To investigate whether other compounds had similar or more hepatoprotective activity than butein, the hepatoprotective effects of thirteen chalcone derivatives (Fig. 2) for D-galactosamine (D-GalN) and lipopolysaccharide (LPS)-induced fulminant hepatic failure in mice were determined. The GalN/LPS model provides a practical tool for the evaluation of drugs that interfere with hepatic apoptosis as well as inflam-
MATERIALS AND METHODS

Reagents

Chalcone derivatives were synthesized at the Department of Medicinal Chemistry, College of Pharmacy, Yanbian University. The compounds were dissolved in Tween-80. D-Galactosamine (GalN, E.coli 0111:B4) and lipopolysaccharide (LPS, E.coli 0111:B4) was purchased from Sigma-Aldrich (St. Louis, USA). Bifendate (DDB) was provided from a pharmaceutical factory in Guang Zhou, China (Batch number 980612).

Animal and experimental protocol

Male C57BL/6 mice (20–25 g) were purchased from the Laboratory of Animal Research, College of Medicine, Yanbian University, and fed with a normal standard chow Celsius diet and tap water, ad libitum. The mice were housed in plastic cages and maintained under conditions of 25 °C, 50-60% relative humidity, and 12 h lightdark cycles throughout the experiment. The mice were maintained in these facilities for at least one week prior to the experiment.

Chalcone derivatives preparations were administered intraperitoneally or orally to mice at 12 h and 1 h before GalN/LPS administration. The doses of the chalcone derivatives preparations administered are shown in Table I. An hour after the second chalcone derivative treatment, mice were given an intraperitoneal injection of GalN (700 mg/kg body weight), immediately followed by an intraperitoneal injection of LPS (10 μg/kg body weight) to induce fulminant hepatitis. Serum and liver tissue samples were obtained 1 h and 8 h after administration of GalN/ LPS. There were at least 10 animals in each group.

Animal experiments were carried out under the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999. The animal care committee of our institution approved the present study.

Blood biochemistry and serum TNF-alpha assay

Blood was collected at 1 h and 8 h after GalN/LPS administration. Serum levels of AST and ALT at 8 h after GalN/LPS injection were quantified using an Autodry Chemistry Analyzer (SPOTCHEM™ SP4410, Arkray, Japan). Serum TNF-alpha level was determined using Quantikine® Mouse TNF-alpha Immunoassay kit (R&D, Minneapolis, USA) at 1 h after GalN/LPS injection according to the manufacturer's protocol.

Determination of lethality

Chalcone derivative preparations were administered intraperitoneally at a dose of 10 mg/kg to mice at 12 h and 1 h before GalN/LPS injection. The numbers of dead mice were counted 24 h after GalN/LPS injection.

Histopathological analysis

The liver, fixed in formalin solution, was embedded in paraffin, 4-μm sectioned, stained with hematoxylin-eosin and photographed at 100 × magnification. Apoptotic bodies and nuclei displaying chromatin condensation were observed and compared between each treated group.

DNA fragmentation analysis

Liver tissue was obtained 8 h after GalN/LPS injection. Genomic DNA was extracted from liver tissues using a Wizard® genomic DNA purification kit (Promega, Madison, USA) according to the manufacturer’s protocol. Extracted DNA was subjected to electrophoresis on a 2% agarose gel containing 0.1 μg/mL ethidium bromide.