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Protective effects of echinacoside on carbon tetrachloride-induced hepatotoxicity in rats

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Abstract

The aim of this study was to investigate the possible protective effects of echinacoside, one of the phenylethanoids isolated from the stems of *Cistanches salsa*, a Chinese herbal medicine, on the free radical damage of liver caused by carbon tetrachloride in rats. Treatment of rats with carbon tetrachloride produced severe liver injury, as demonstrated by dramatic elevation of serum ALT, AST levels and typical histopathological changes including hepatocyte necrosis or apoptosis, haemorrhage, fatty degeneration, etc. In addition, carbon tetrachloride administration caused oxidative stress in rats, as evidenced by increased reactive oxygen species (ROS) production and MDA concentrations in the liver of rats, along with a remarkable reduction in hepatic SOD activity and GSH content. However, simultaneous treatment with echinacoside (50 mg/kg, intraperitoneally) significantly attenuated carbon tetrachloride-induced hepatotoxicity. The results showed that serum ALT, AST levels and hepatic MDA content as well as ROS production were reduced dramatically, and hepatic SOD activity and GSH content were restored remarkably by echinacoside administration, as compared to the carbon tetrachloride-treated rats. Moreover, the histopathological damage of liver and the number of apoptotic hepatocytes were also significantly ameliorated by echinacoside treatment. It is therefore suggested that echinacoside can provide a definite protective effect against acute hepatic injury caused by CCl_4 in rats, which may mainly be associated with its antioxidative effect.

Keywords: Carbon tetrachloride; Oxidative stress; Acute liver injury; ROS; Echinacoside

1. Introduction

Drug/chemicals-induced liver injury, also known as toxic hepatopathy, is a major clinical problem. It is showed by epidemiological studies that the prevalence of drug/chemicals-induced hepatopathy went up over the past decades especially in the developing countries (Bissel et al., 2001; Larrey, 2000; Lee, 2003). Despite that extensive studies have been made for decades, the precise mechanisms underlying drug/chemicals-induced liver injury are still unclear and remain controversial. And there is still lack of effective therapeutic strategies or specific medicines for such liver diseases. Recent studies indicated that oxidative stress might be a pivotal originating factor in the pathogenesis of the liver diseases including drug-induced hepatic damage, alcoholic hepatitis, and viral hepatitis or ischemic liver injury (Albano, 2002; Amin and Hamza, 2005; Jaeschke et al., 2002). Over production of free radicals are toxic to hepatocytes and initiate reactive oxygen species (ROS)-

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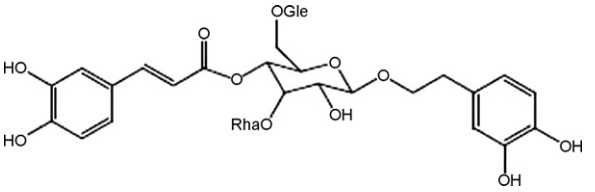

Fig. 1. Chemical structure of echinacoside.

mediated cascade causing hepatocyte death, leading to acute hepatic damage (Jaeschke, 2000; Gutteridge, 1993; Pessayre, 1995; Sies, 1991). Therefore, antioxidative treatment was proposed to be a potential means of preventing or attenuating toxic liver injury (Hensley et al., 2000; Higuchi and Gores, 2003; Kaplowitz, 2002). However, the effects of most antioxidants against acute liver damage are not satisfactory enough to apply into clinical situations.

Echinacoside (Fig. 1) is one of the phenylethanoids isolated from the stems of *Cistanches salsa*, a Chinese herbal medicine, which is an important crude drug used both as an antisenium and antifatigue agent (Deng et al., 2004; Xiong et al., 1996). Several phenylethanoids have been shown to possess free radical scavenging properties and protect oxidative stress-induced toxic injuries (Deng et al., 2004; Gao et al., 1999). In the present study we aimed to investigate the potential effects of echinacoside in reducing oxidative stress, and improving histopathological abnormality in the liver of rats treated with CCl_4 that not only could provide some helpful information for the therapy or prevention of such liver disease, but might promote the understanding of its mechanisms, especially the role of free radicals or oxidative stress in the pathogenesis of acute toxic liver injury.

2. Materials and methods

2.1. Chemicals and reagents

Echinacoside from *C. salsa* was kindly supplied by Peking University Modern Research Center for Traditional Chinese Medicine. The purity of the compounds was shown to be more than 95% on high-performance liquid chromatography (HPLC). The TUNEL assay kit was obtained from Roche Diagnostics Company (Germany). PBN was purchased from Sigma Chemical Co., USA. All other reagents or drugs were of analytical grade.

2.2. Animals and treatments

Twenty-four adult male Sprague–Dawley rats weighing 200–250 g obtained from Peking University Animal Breeding Unit were used in this study. All animals were kept under the same laboratory conditions of temperature ($25 \pm 2\,^{\circ}C$) and lighting (12:12 h light:dark cycle) and were given free access to standard laboratory chow and tap water. All rats were allowed to acclimatize for 1 week prior to experimentation. All experimental procedures involving animals were approved by the ethical animal committee of Peking University, Beijing, PR China.

The animals were randomly divided into three groups containing eight rats in each. Group 1 served as controls and received an injection of vehicle (olive oil) alone; group 2 was injected intraperitoneally (i.p.) with CCl_4 dissolved in an equal volume of olive oil at a dose of 3 ml/kg, which is well documented to induce hepatotoxicity. Echinacoside was dissolved in physiological saline and then administered to group 3 at a dose of 50 mg/kg body weight, followed by CCl_4 treatment to induce hepatic damage in rats. The dose of echinacoside adopted in this study was based on the preliminary studies in our research group.

2.3. Sample collection and hepatic function assays

Before the rats were killed, they were fasted for 24 h after administration of CCl_4. At the end of the experiment, all animals were sacrificed to collect blood samples, which were allowed to clot and centrifuged at $3000 \times g$ for 10 min to obtain serum. All serum samples were sterile, haemolysis-free and were kept at $-70\,^{\circ}C$ before determination of the biochemical parameters. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) as markers of hepatic function were measured by using a multiparameteric analyzer (AU 5400, Olympus, Japan).

2.4. Measurement of MDA, SOD and GSH in the liver of rats

Part of the liver tissue was prepared to make homogenization with a buffer containing 0.15 M KCl to obtain 1:10 (w/v) homogenates. Homogenates were then centrifuged at $12{,}000 \times g$ ($4\,^{\circ}C$) for 20 min to collect the supernatant for determination of MDA, GSH concentrations and SOD activity (Wen et al., 2006). Protein concentrations were measured according to the Bradford method (Bradford, 1976).

MDA, the last product of lipid breakdown caused by oxidative stress, was evaluated by the thiobarbituric acid reactive substances method (TBARS) and was expressed as nmol/mg protein (Draper and Hadley, 1990).

Glutathione (GSH) concentration was measured by a kinetic assay using a dithio-nitrobenzoic acid recycling method and was expressed as μmol/g protein (Gutteridge and Halliwell, 1990).

Superoxide dismutase (SOD) activity was estimated by a method based on the production of H_2O_2 from xanthine by xanthine oxidase and reduction of nitroblue tetrazolium (Sarban et al., 2005). The product was evaluated spectrophotometrically at 560 nm and expressed as U/mg protein.