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Effects and mechanisms of crude astragalosides fraction on liver fibrosis in rats

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Abstract

Astragalosides is the major active constituent of Radix Astragali. The present study was carried out to investigate the effect of crude astragalosides fraction (CAF) on rats liver fibrosis and its possible mechanisms. Hepatic fibrosis was induced by subcutaneous injection with 50% CCl_4 in Sprague–Dawley rats. The amount of CCl_4 administered was 1 mg kg^{-1}. The alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels in plasma and hydroxyproline (Hyp), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) contents in liver tissue were assayed by spectrophotometry. The hyaluronic acid (HA) and procollagen III (PC III) were assessed by radioimmunoassay. Tumor necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β1) levels in culture supernatants of Kupffer cells (KCs) were determined with ELISA. Liver samples collected after 8 weeks of CCl_4 treatment were stained with hematoxylin–eosin (HE) and massion, and scored. Intragastric administration of CAF (10, 20 and 40 mg kg^{-1}) significantly decreased indices of liver and spleen, the serum transaminase activities, HA and PC III levels, and Hyp and MDA contents in liver tissue in rats of hepatic fibrosis. Decreased SOD and GSH-px levels were reversed after administration of CAF. Histopathological scores showed CAF had inhibitory effect on the progression of hepatic fibrosis. In the in vitro experiments, CAF significantly reduced TNF-α and TGF-β1 levels in culture supernatants of KCs. The results showed CAF significantly inhibited the progression of hepatic fibrosis induced by CCl_4, and the inhibitory effect of CAF on hepatic fibrosis might be associated with its ability to scavenge free radical and inhibit the production of TNF-α and TGF-β1 from activated KCs.

Keywords: Crude astragalosides fraction; Radix Astragali; Liver fibrosis; Free radicals; Tumor necrosis factor-α; Transforming growth factor-β1

1. Introduction

Hepatic fibrosis is a dynamic process caused by chronic liver injury due to various etiologies (viral infections, alcohol abuse, and metal overload), eventually leading to cirrhosis. It is predominantly characterized by excessive accumulation of extracellular matrix (ECM) caused by both an increased synthesis and decreased or unbalanced degradation of ECM. The accumulation of ECM proteins distorts the hepatic architecture by forming a fibrous scar, and the subsequent development of nodules of regenerating hepatocytes defines cirrhosis. Cirrhosis produces hepatocellular dysfunction and increased intrahepatic resistance to blood flow, which result in hepatic insufficiency and portal hypertension, respectively (Gines et al., 2004). There is no standard treatment for liver fibrosis. Although there are a few anti-inflammatory and anti-fibrotic drugs showing effectiveness on treating hepatic fibrosis, their side effects and toxicity call for new and more effective natural drugs.

Radix Astragali (root of *Astragalus*; Huangqi) is a popular traditional Chinese medicine, and *Astragalus membranaceus* and *Astragalus membranaceus* var. *mongolicus* are two commonly used species. Astragalosides, extracted from the root of *Astragalus membranaceus*, is the active compound, in addition to astragalus polysaccharides. Investigations revealed that Radix Astragali has protecting liver lesion and anti-fibrotic properties (Cheng et al., 2000; Zhang et al., 1992). In vitro, Radix Astra-

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gali, its extracts, and its active ingredients such as astragalosides, astragalus polysaccharides could inhibit hepatic stellate cell proliferation and collagen production (Wu et al., 2003). Astragalosides is the major active constituents of Radix Astragali, which consist of astragalosides I–IV, soyasaponin, etc. However, the effect of astragalosides on CCl_4-induced rats liver fibrosis in vivo and its mechanism of action are unknown and need further investigation. The present study was designed to investigate the effect of crude astragalosides fraction (CAF) on rat's hepatic fibrosis induced by CCl_4. In addition, the effect of CAF on tumor necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β1) from Kupffer cells (KCs) was also evaluated.

2. Materials and methods

2.1. Reagents

Radix Astragali was purchased from Hefei Heyitang Pharmacy, China. The production place of *Astragalus membranaceus* (Fisch.) Bge. was Shanxi Province (China). The specimen was identified by Professor Lumin Pan in Department of Medicinal Plant, School of Pharmacy, Anhui College of TCM. CCl_4 was purchased from Beijing Chemical Factory (China). The kits of alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) were purchased from Nanjing Jiancheng Institute of Biotechnology (China). The procollagen type III (PC III) and hyaluronic acid (HA) radioimmunoassay kits were purchased from Shanghai Navy Medical Institute (China). ELISA kits of TNF-α and TGF-β1 were obtained from Sigma Chemical (St. Louis, MO). The other chemicals used were reagent grade from commercial source.

2.2. Preparation of CAF

Sliced dried roots (15 kg) were extracted three times with 80% aqueous ethanol, 2 h each time. Then, the extracts were combined and evaporated to dryness under reduced pressure, which yielded 2.7 kg of dry powder. About 1 kg of the residue obtained from the combined extract was dissolved with 6 L of water. After filtration, the aqueous solution was extracted three times with 8 L water-saturated *n*-butanol successively each time, which yielded 231.2 g dry powders after being combined and evaporated to dryness under reduced pressure. Then, the *n*-butanol extract was chromatographed on polystyrene resin (D101, 0.3–1.25 mm; Nankai Chemical Factory, Tianjin, China) with water, 40% ethanol and 70% ethanol, respectively. Portions of 70% ethanol were collected, and evaporated to dryness under reduced pressure, which yielded 31.2 g of dry powder. The dry powder contained 76.3% CAF by chromatometry. The CAF consist of astragaloside I–IV, soyasaponin, etc. Astragaloside IV, accounting for 15.2% by high-performance liquid chromatography–evaporative light scattering detector method analysis, was one of the main effective components of CAF. Before use, CAF was dissolved in 0.5% sodium carboxymethylcellulose (CMC-Na) solutions. To obtain the dose of 10, 20, or 40 mg kg^{-1} body weight, 1, 2, or 4 mg ml^{-1} CAF solution was intragastrical administered. The amounts of CAF was 1 ml/100 g body weight.

2.3. Animals and treatment

Male Sprague–Dawley rats weighing 150–180 g were used. All animals were housed in conventional cages with free access to water and rodent chow at 20–22 °C with 12 h light–dark cycle. All procedures involving the use of laboratory animals were in accordance with National Institutes of Health guidelines. Rats were randomly divided into five groups, which included control group, model group and CAF (10, 20 and 40 mg/kg) group. The procedure for CCl_4-induced model of liver fibrosis was based on the method of Iredale et al. (1998), with some modifications. Rats were subcutaneously injected with 50% CCl_4 mixed with vegetal oil, twice a week for 8 weeks. The amount of CCl_4 administered was 1 mg kg^{-1}. At the beginning of injection of CCl_4, the CAF was administered by intragastric injection (ig) at doses of 10, 20 and 40 mg kg^{-1} daily for 8 weeks. The control group was administered with the same volume of vehicle.

At 24 h after final injection of CCl_4, a laparotomy was performed and blood was drawn from the abdominal aorta under ether anesthesia, after which the animals were killed and the liver and spleen promptly were removed, and weighed. The serum was stored at −70 °C after separation until assayed as described below. The tissues were also stored at −70 °C until required.

2.4. Serum fibrosis markers, ALT and AST levels

The levels of PC III and HA in the serum were assayed with radioimmunoassay. The activities of ALT and AST were determined following the kit instructions.

2.5. Hydroxyproline (Hyp) content in the liver

Liver tissue was weighed, hydrolyzed in HCl, and analyzed for total Hyp content, according to the method of Mitchell and Taylor (1970) with minor modifications.

2.6. Measurement of MDA, SOD, and GSH-px in liver homogenate

Portion of the liver were homogenized with Tris–HCl (5 mmol L^{-1} containing 2 mmol L^{-1} EDTA, pH 7.4). Homogenates were centrifuged (1000 × *g*, 10 min, 4 °C) and the supernatant was used immediately for the assays of MDA, GSH-px, and SOD following the kit instructions. In brief, MDA in liver tissue was determined by the thiobarbituric acid method. The assay for total SOD and GSH-px were based on its ability to inhibit the oxidation of oxyamine by the xanthine–xanthine oxydase system.

2.7. KCs isolation and experimental protocol

KCs were isolated by in situ collagenase perfusion and purified by Nycodenz density gradients as previously described