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Antifibrotic activity of galangin, a novel function evaluated in animal liver fibrosis model

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ABSTRACT

This study aimed to investigate the effects of galangin on liver fibrosis in rats induced by subcutaneous injection of carbon tetrachloride (CCl_4). The administration of CCl_4 to rats for 12 weeks caused significant increase of hyaluronic acid, laminin, alanine transaminase, aspartate transaminase and decrease of total protein, albumin in serum, while the influences could be reversed by galangin. Galangin markedly reduced hepatic malondialdehyde, hydroxyproline concentration, increased activities of liver superoxide dismutase, glutathione peroxidase compared with CCl_4-treated rats. Histological results indicated that galangin alleviated liver damage. In addition, treatment with galangin significantly down-regulated expressions of α-smooth muscle actin and transforming growth factor β1. These results suggest galangin can inhibit liver fibrosis induced by CCl_4 in rats, which was probably associated with its effect on removing oxygen free radicals, decreasing lipid peroxidation, as well as inhibiting hepatic stellate cells activation and proliferation.

1. Introduction

Fibrosis in liver can be caused by a variety of pathological factors, including hepatitis viruses (particularly hepatitis B and C viruses), alcohol and drug abuse, metabolic diseases due to overload of iron or copper and autoimmunity against hepatocytes or bile duct epithelium (Friedman, 2000). Advanced liver fibrosis eventually leads to cirrhosis and liver failure, for which no effective treatments other than liver transplantation are currently available (Capasso and Mascolo, 2003; Li et al., 2012). Therefore, prevention of liver fibrosis is a crucial step for protecting the liver against the occurrence of cirrhosis and failure (Yang et al., 2010).

Liver fibrosis, with cirrhosis in particular, is typically associated with significant morbidity and mortality, and is characterized by imbalance between the extracellular matrix synthesis and degradation. During the activation process, hepatic stellate cells (HSCs) undergo phenotype transformation from vitamin-A-storing quiescent cells to myofibroblast-like activated cells (Friedman, 2008). As a major source of collagen type I, activated HSCs secrete fibrillar collagens, resulting in the deposition of fibrotic matrix (Henderson and Iredale, 2007). Although reactive oxygen species (ROS) at physiological conditions function as signaling molecules to mediate and maintain normal activities in organisms, excess ROS have been related to many pathological conditions. Increased ROS generation, such as superoxide anion,

Abbreviations: α-SMA, α-smooth muscle actin; ALB, albumin; ALT, alanine transaminase; AST, aspartate transaminase; CCl_4, carbon tetrachloride; CMC-Na, carboxymethyl cellulose-Na; ECM, extracellular matrix; GSH-Px, glutathione peroxidase; HA, hyaluronic acid; HI, hepatic index; HSCs, hepatic stellate cells; Hyp, hydroxyproline; LN, laminin; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; TGF-β1, transforming growth factor β1; TP, total protein.

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hydrogen peroxide, and hydroxyl radical has been implicated in the progression of chronic liver diseases via stimulate HSCs proliferation.

Certain several herbal medicines have shown to have protective effects from liver fibrosis and injuries (Hsieh et al., 2008). Flavonoids, which have shown a wide effect on biological systems, constitute one of the largest groups of natural secondary metabolites ubiquitous in all vascular plant (Capasso and Mascolo, 2003). Galangin, a member of the flavonol class of flavonoids, is primarily present in the rhizome of the *Zingiberaceae* family's *Alpinia* galanga plant and honey. Modern pharmacological studies have shown that galangin has several pharmacological effects such as anti-clastogenic (Heo et al., 2001), antiinflamatory (Toit et al., 2009) and antibiotic (Eumkeb et al., 2010). Due to its low toxicity and potent pharmacological activities, galangin has become a promising research and development target. Despite a large amount of data is available on the various effects of galangin, little is known about this effect on liver fibrosis.

CCl_4 intoxication is frequently used to produce oxidative stress and chemical liver injuries. CCl_4 is a well-known hepatotoxicant model that is activated by cytochrome P-450 and initiates oxidative and biochemical stress that ultimately damage liver and other tissues, including kidney, heart, lung, testis, brain and blood (Szymonik-Lesiuk et al., 2003; Fan et al., 2009). Because the specific treatments to prevent progressive fibrosis of the liver are not available, the present study was performed to investigate the therapeutic effect of galangin on composite factor-induced liver fibrosis and to explain its possible mechanisms of action.

2. Materials and methods

2.1. Materials

Colchicine was obtained from Kunming Chemical Co., Inst, China. Carbon tetrachloride was purchased from Shantou Chemical Co., Inst, China. Galangin (3,5,7-trihydroxyflavone) was purchased from Hangzhou Eastbiopharm Co., Ltd. (Hangzhou, China). Peanut oil was purchased from Jinhai Grain and Oil Industrail Co., Ltd. (Qinhuangdao, China). Hyaluronic acid (HA) and laminin (LN) kits were obtained from Beijing North Institute of Biological Technology (Beijing, China). Alanine transaminase (ALT), aspartate transaminase (AST), total protein (TP), albumin (ALB), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and hydroxyproline (Hyp) kits were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Animals

Sixty healthy six-week-old male Sprague-Dawley rats weighing 200 ± 20 g were obtained from Nanjing Medical University (Nanjing, China). The animals, housed in controlled environmental conditions (24 ± 2 °C temperature, 60–70% relative humidity, 12 h light/dark cycle), were provided standard pellet diet and water ad libitum. All procedures were conducted in accordance with the Guidelines on the Care and Use of Laboratory Animals (Chinese Council on Animal Research and the Guidelines of Animal Care). The study was approved by the Ethical Committee of China Pharmaceutical University.

2.3. Experimental protocol

The animals were acclimatized for 2 days and divided into six groups of ten animals each: the normal control group, the model group, the positive control group (colchicin 0.2 mg/kg) and three galangin groups (20 mg/kg, 40 mg/kg and 80 mg/kg respectively). The doses were selected based on the observations of our preliminary study (unpublished data). The rat model of liver fibrosis induced by carbon tetrachloride was conducted using the method developed by Zhang et al. (2011) with minor modifications. Animals were given intraperitoneally (*i.p.*) CCl_4 dissolved in peanut oil (4:6, V/V), with a dose of 2 ml/kg, twice a week for 12 weeks, except the normal control group which received vehicle only. Meanwhile, the treatment groups were given the corresponding drugs, which were dissolved in 0.5% CMC-Na, every day via gavage administration (10 ml/kg). The normal control group and model group were given 0.5% CMC-Na by the same way with the same method. 48 h after the last administration, various tissue samples were taken for histopathological examination and analysis.

2.4. Serum biochemistry assays

HA and LN were assayed by radioimmunoassay according to the manufacturer's instructions (North Institute of Biological Technology, Beijing, China). Measurements of ALT, AST, TP and ALB were carried out according to the instructions of the kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Hepatic lipid peroxidation and hydroxyproline assays

Liver tissues were homogenized on ice with tris–HCl (5 mmol/L containing 2 mmol/L EDTA, pH 7.4), centrifuged at $1000 \times g$ for 15 min at 4 °C. Supernatants were collected for further analyses. SOD, GSH-Px, MDA and Hyp in homogenized fresh liver samples were determined according to the kit instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.6. Histopathological examination

Liver samples were fixed in formalin, embedded in paraffin, and routinely stained by hematoxylin and eosin (H&E). Individual sections were examined independently, and without prior knowledge of the experiment details, for evidence of fibrosis.

2.7. Immunohistochemical determination

Fresh livers tissues taken from the left lobe of each rat were fixed with 4% paraformaldehyde for 24 h, followed by immunohistochemical stainings for α-smooth muscle actin (α-SMA) and transforming growth factor β1 (TGF-β1) were assessed by the routine immunohistochemistry SP method,