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Effects and mechanisms of extract from *Paeonia lactiflora* and *Astragalus membranaceus* on liver fibrosis induced by carbon tetrachloride in rats

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Abstract

Paeonia lactiflora and *Astragalus membranaceus* are two popular traditional Chinese medicines, commonly used in Chinese herb prescription to treat liver disease. The extract prepared from the roots of *Paeonia lactiflora* and *Astragalus membranaceus* (PAE) demonstrated more excellent hepato-protective activity than the single herbs used individually as indicated in our preliminary studies. The present study was carried out to investigate the effects of PAE on liver fibrosis in rats induced by carbon tetrachloride (CCl_4) and to explore its possible mechanisms. Liver fibrosis was induced in male Sprague–Dawley rats by injection with 50% CCl_4 subcutaneously twice a week for 8 weeks. At the same time, PAE (40, 80 and 160 mg/kg) was administered intragastrically. Upon pathological examination, the PAE-treated rats significantly reduced the liver damage and the symptoms of liver fibrosis. Administration of PAE decreased CCl_4-induced elevation of serum transaminase activities, hyaluronic acid, laminin and procollagen type III levels, and contents of hydroxyproline in liver tissue by approximately 30–60%. It also restored the decrease in SOD and GSH-Px activites and inhibited the formation of lipid peroxidative products during CCl_4 treatment. Moreover, PAE (80, 160 mg/kg, ig) decreased the elevation of TGF-β1 by 47.7% and 53.1%, respectively. In the primary cultured hepatic stellate cells (HSCs), PAE also significantly decreased [^{3}H] thymidine incorporation in cells stimulated with platelet-derived growth factor-B subunit homodimer (PDGF-BB) and suppressed [^{3}H] proline incorporation. These results suggested that PAE significantly inhibited the progression of hepatic fibrosis induced by CCl_4, and the inhibitory effect of PAE on hepatic fibrosis might be associated with its ability to scavenge free radicals, decrease the level of TGF-β1 and inhibit collagen synthesis and proliferation in HSCs.

Keywords: *Paeonia lactiflora*; *Astragalus membranaceus*; Liver fibrosis; Oxidative stress; Transforming growth factor-β1; Hepatic stellate cell

1. Introduction

Liver fibrosis represents a major medical problem with significant morbidity. Hepatitis viral infections, including hepatitis B and hepatitis C (especially hepatitis B in China), represent the major cause of liver fibrosis. Other stimuli for liver fibrosis include drug-induced, helminthic infection, autoimmune disorders, iron or copper overload and biliary obstruction. Fibrosis can be classified as a wound healing response to a variety of chronic stimuli. It is characterized by an excessive deposition of extracellular matrix proteins of which type I collagen predominates. This excess deposition of extracellular matrix proteins disrupts the normal architecture of the liver that alters the normal function of the organ, resulting in pathophysiological damage to the organ (Bataller and Brenner, 2005; Tsukada et al., 2006). Hepatic stellate cells (HSCs) are presently regarded as one of the key cell types involved in the progression of liver fibrosis. The activation of HSCs to a proliferative, myofibroblastic phenotype plays a key role in hepatic fibrogenesis, since these cells are the principal cellular source of the excess collagen synthesis during hepatic fibrosis (Friedman, 2004). If left untreated, fibrosis can progress to liver cirrhosis ultimately leading to organ failure and death. Current evidence indicates that hepatic fibrosis

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even cirrhosis is dynamic and can be bidirectional (involving phases of progression and regression) (Pinzani and Rombouts, 2004). Efforts have been made to search for effective anti-fibrotic agents. However, no effective antifibrotic therapies are available until now. Therefore, the prevention of liver fibrosis has a very great significance both in theory and in practice.

Medicinally, natural drugs have made a significant contribution to the treatment of liver fibrosis. Use of herbal drugs in the treatment of liver diseases has a long tradition, especially in Eastern medicine. Examples could be found in 'Yellow Emperor's Internal Classic', a highly systematic and comprehensive ancient Chinese medical text dated back to 475–221 B.C. (Schuppan et al., 1999; Chor et al., 2005). Herbal medicines used in China are now being manufactured as drugs containing ingredients of standardized quality and quantity. Traditional Chinese medicine treatment is based on overall analysis of symptoms and signs, as well as the physical condition of the patient. Nowadays, practitioners of traditional Chinese medicine routinely use herbs to treat chronic liver disease and cirrhosis. *Paeonia lactiflora* pall root, a traditional Chinese herb, has been used to relieve the pain and has been a component of effective prescriptions for treatment of liver disease (Dai et al., 1993). Total glucosides of peony (TGP) which consist of paeoniflorin, albiflorin, benzoylpaeoniflorin, oxypaeoniflorin, paeonin, etc., extracted from *Paeonia lactiflora* pall root, have been recognized as the valuable traditional herbs used in the treatment of rheumatoid arthritis, systemic lupus erythematosus and hepatitis with a long history in traditional Chinese medicine (Zhang et al., 2001). The anti-inflammatory, anti-oxidative, anti-hepatic injury and immunoregulatory activities of TGP have been extensively proved in our laboratory for many years. Previous studies demonstrated that TGP could retard the progression of experimental immunological hepatic fibrosis through inhibition of collagen synthesis and decreasing oxidative stress, but had modest effect on HSCs proliferation (Wang et al., 2005). *Astragalus membranaceus* is a popular traditional Chinese herb. It is used to replenish the vital energy for the treatment of lacking strength, anorexia and loose stools, spontaneous sweating and chronic nephritis with edema and proteinuria (Pharmacopoeia of the People's Republic of China, 2005 edition). The modern pharmacological studies revealed that Radix Astragali possessed immunostimulant and anti-fibrotic properties (Cheng et al., 2000; Lee et al., 2003). In vitro studies in our laboratory demonstrated that Radix Astragali, its extracts, and its active ingredients such as astragalosides, astragalus polysaccharides could significantly inhibit HSCs proliferation and collagen production (Wu et al., 2003). We have sought a preparation that has the ability to reduce matrix synthesis as well as affecting HSCs proliferation. In traditional Oriental medicine, it is conventional to combine different herbs in order to achieve a variety of treatment purposes simultaneously, or to enhance a single effect without causing severe side effects (Nishiyama et al., 1995). In order to obtain a more effective remedy for the treatment of liver fibrosis other than exclusively using *Paeonia lactiflora* or *Astragalus membranaceus*, we combined these two herbs based on both traditional references and the results of our previous work mentioned above. As a result of preliminary tests of extracts with several different combination-ratios (data not shown), we found that the combination in the ratio of 4:1 of two plants, *Paeonia lactiflora* roots and *Astragalus membranaceus* roots, respectively, exhibited the most significant hepato-protective activity among the combinations tested. Standardized extract of the Chinese herb prescription composed of *Paeonia lactiflora* and *Astragalus membranaceus* (PAE) may be expected to synergistically exert the antifibrotic effects based on findings from our previous reports. PAE was mainly composed of the total glucosides of paeony and the total astragalosides. Our previous studies have shown that PAE has protective effects on chemical liver injury in mice through inhibiting oxidative stress (Wu et al., 2006). These studies have resulted in considerable interest in PAE as a therapeutic agent in chronic liver disease. To further evaluate the antifibrotic activity of PAE, the present study was designed to investigate the effects of PAE administration on carbon tetrachloride (CCl_4)-induced rats liver fibrosis in vivo. Furthermore, the actions of PAE on markers of oxidative stress and fibrogenesis were investigated. In addition, the effects of PAE on proliferation and collagen synthesis in cultured rat HSCs were evaluated in vitro.

Colchicine is an alkaloid agent that has been widely used in clinical practice for the treatment of acute gout and other immunologic diseases. Long-term colchicine treatment in patients with hepatic fibrosis appears to exert an anti-inflammatory, anti-fibrotic and immunomodulatory effect (Nikolaidis et al., 2006). In experimental studies, colchicine reduces acute liver injury (Mourelle et al., 1988), inhibits collagen secretion, and increases collagen degradation, thereby it reduces liver fibrosis (Poo et al., 1993). In this study, colchicine was used as a suitable positive control.

2. Materials and methods

2.1. Reagents

Radix Paeonia lactiflora and Radix Astragali were purchased from Hefei Heyitang Pharmacy, China. The production areas of *Paeonia lactiflora pall* and *Astragalus membranaceus* (Fisch.) Bge. were Anhui Province and Shanxi Province(China) respectively. Voucher specimen (Pan 2004002 and Pan 2004013) were identified by Professor Lumin Pan in Department of Materia Madica, School of Pharmacy, Anhui College of Traditional Chinese Medicine and deposited in the Chinese Materia Madica Specimen Center of Anhui College of Traditional Chinese Medicine. Colchicine was obtained from Sigma Chemical Co. (St. Louis, MO, USA). CCl_4 was purchased from Shanghai Xinzhong Chemical Factory (China). Commercial kits used for determining aspartate aminotransferase (AST), alanine aminotransferase (ALT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were obtained from Nanjing Jiancheng Institute of Biotechnology (China). The hyaluronic acid (HA), laminin (LN) and procollagen type III (PC III) radioimmunoassay kits were purchased from Shanghai Navy Medical Institute (China). ELISA kits of transforming growth factor-beta1 (TGF-β1) were obtained from Sigma Chemical (St. Louis, MO). Dulbecco's