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Paridis Rhizoma Sapoinins attenuates liver fibrosis in rats by regulating the expression of RASAL1/ERK1/2 signal pathway

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ABSTRACT

Ethno-pharmacological relevance: Paridis Rhizoma is a Chinese medicinal herb that has been used in liver disease treatment for thousands of years. Our previous studies found that Paridis Rhizoma saponins (PRS) are the critical components of Paridis Rhizoma which has good liver protection effect. However, the anti-hepatic fibrosis effect and the mechanism of PRS have seldom been reported.
Aim of the study: To investigate the potential of PRS in the treatment of experimental liver fibrosis and the underlying mechanism.
Materials and methods: The chemical feature fingerprint of PRS was analyzed by UPLC-PDA. A total of 40 Male Sprague–Dawley (SD) rats were randomly divided into the control group, the model group, the PRS high dose group (PRS H) and the PRS low dose group (PRS L) with 10 rats in each group. The model, PRS H and L groups as liver fibrosis models were established with carbon tetrachloride (CCl_4) method. PRS H and L groups were adopted PRS (300 and 150 mg/kg d^{-1}) treatment since the twelfth week of modeling till the sixteenth week. Pathological changes in hepatic tissue were examined using hematoxylin and eosin (H&E) and MASSON trichrome staining. Immunohistochemical analysis was performed to determine the protein expression of the RASAL1. RT-PCR and western blotting were used to detect the expression of ERK1/2 mRNA and protein.
Results: Four saponins in PRS were identified from 19 detected chromatographic peaks on UPLC-PDA by comparing to the standard compounds. PRS can improve the degeneration and necrosis of hepatic tissue, reduce the extent of its fibrous hyperplasia according to H&E and MASSON staining detection. As was detected in PRS H and L groups, PRS down-regulated p-ERK1/2 mRNA and RASAL1 protein, and up-regulated the level of p-ERK1/2 mRNA and RASAL1 protein.
Conclusion: These results demonstrated that PRS can attenuate CCl_4-induced liver fibrosis through the regulation of RAS/ERK1/2 signal pathway.

1. Introduction

Liver Fibrosis is a consequence of almost all chronic liver diseases predominantly arising from hepatitis B virus (HBV), alcohol liver disease, hepatitis C virus (HCV), autoimmune liver disease, primary biliary cirrhosis (PBC), fatty liver, etc. (Lee, 1997; Elizabeth and Derek, 2012). Liver fibrosis is a common pathological process for the majority of liver diseases which in a significant minority of patients leads to end stage cirrhosis and/or hepatocellular carcinoma (HCC) (Elizabeth et al., 2012). One caveat is that liver cirrhosis and its complications are the main factors of death in chronic liver disease (Organization W H, 2015). Also, it has become a worldwide health problem because the mortality of infection in cirrhosis is still high and has not changed substantially (Friedman, 2003; Afdhal and Curry, 2007).

Although chronic hepatitis has a diverse etiology, development of hepatic fibrosis is a common outcome and cirrhosis is considered to be the universal end-stage of chronic liver disease (van der Meer et al., 2014). The formation and development of liver fibrosis crucially affect prognosis and outcome of the chronic liver diseases, and treatment of liver fibrosis is one of the most challenging tasks among the clinical treatment of chronic liver diseases. Fortunately, evidence has accumulated to suggest that

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hepatic fibrosis (HF) is the middle stage of chronic liver disease development to liver cirrhosis and can be reversed if the underlying cause of liver injury is taken care of Duseja (2013) and Okabe et al. (2015).

Traditional Chinese Medicine (TCM) has been used to widely treat chronic liver diseases and its therapeutic benefits have been recognized for centuries. Recently, many controlled trials have been done to investigate their efficacy (Zeng et al., 2011) and the results indicated that more than 20 compounds and extracts from Chinese medicines have been reported to have liver protective and antifibrotic effects (Qi et al., 2013). Various studies on their chemistry and pharmacology as well as clinical trials have been done, although their typical mechanisms remain unclear (Ju et al., 2014; Zhou et al., 2014).

Paris polyphylla var. *yunnanensis* (Franch.) Hand.-Mazz., commonly known as Paridis Rhizoma in China was documented in the "Chinese pharmacopoeia" 2010 Edition. It was first recorded in "Shennong Herb" named as Zaoxiu. The TCM experience in clinical think Paridis Rhizoma have the effect of "clearing away heat and toxic", "promoting blood circulation and removing blood stasis" which has been used in treatment of hepatitis B for many years (Ning and Cui, 2008; Qiao and Zhang, 2008). The dried Paridis Rhizoma has been used to treat fractures, parotitis, hemostasis, snake bite, and abscess in folk medicines for thousands of years. In addition, it has played an important role in the medicine development for anti-tumor, immunity adjustment, analgesia, and anti-inflammation (He and Li, 2012).

It is reported that Paridis Rhizoma Sapoinins (PRS) are the main active components in Paridis Rhizoma (Han et al., 2012). Our previous study and literature report revealed that PRS has good liver protection and anti-hepatic fibrosis effects (Hong et al., 2014; Man et al., 2014). However, its mechanism is still not clear. In current study, we aimed at the mechanism of PRS on liver fibrosis attenuation in rats. To do this, chemically liver fibrosis rat model was induced by CCl_4, liver tissue histology of the rats were determined by hematoxylin and eosin (H&E) and Masson's trichrome staining, protein expression of RASAL1 (RAS protein activator like 1) were evaluated by immunohistochemical method, mRNA and phosphorylation protein expression of ERK1/2 (extracellular regulated protein kinase) in liver tissue were evaluated by RT-PCR and western-blot method, respectively.

2. Materials and methods

2.1. PRS preparation

The dried rhizomes of Paridis Rhizoma was purchased from Chinese herbal medicine Pharmacy of The First Affiliated Hospital, Anhui University of Chinese Medicine, (Hefei City, Anhui Province, China) and it had been identified by Professor Pen Hua-sheng (Anhui University of Chinese Medicine, Anhui Province, China). Paridis Rhizoma Saponins (PRS) were prepared as previously described (Man et al., 2009a, 2009b) and its yield was 1.15%. The content of PRS in extract were determined spectrophtotometrically using diosgenin as calibration standard at 408 nm wavelength (Yang and Zhang, 2007). The results of quantification indicated that steroidal saponins had high content in PRS (53.22 g steroidal saponins/100 g PRS). In addition, the saponins include polyphyllin VII, polyphyllin VI, polyphyllin II and polyphyllin I in PRS was determined by UPLC-ELSD (Han et al., 2012) in comparison with reference substances and the results indicated that the contents of polyphyllin VII, polyphyllin VI, polyphyllin II and polyphyllin were 2.41%, 3.15%, 2.49% and 9.92%, respectively.

Table 1
Primer sequences and the reaction conditions of ERK1, ERK2 and GAPDH genes.

Gene	Primer sequences (5′-3′)	Fragment length (bp)	Tm (°C)
ERK1	(F): 5′-CCATCCCAAGAGGACCTAAA-3′ (R): 5′- ATCATCCAGCTCCATGTCAA-3′	273	55
ERK2	(F): 5′- CGCGCTACACTAATCTCTCG -3′ (R): 5′-ATCATGGTCTGGATCTGCAA -3′	470	58
GAPDH	(F): 5′- CAAGGTCATCCATGACAACTTTG -3′ (R): 5′- GTCCACCACCCTGTTGCTGTAG -3′	496	55

2.2. Analysis of PRS by UPLC-PDA

Waters Acquity ultra-performance liquid chromatography (UPLC) H-Class system consisting an autosampler and a quaternary pump, thermostatted column compartment and PDA (Waters, Milford, MA) was used for acquiring chromatograms and UV spectra. An Acquity BEH C_{18} (2.1 mm × 100 mm, 1.7 μm; Waters, Milford, MA) analytical column coupled with a column filter were used with column temperature set at 30 °C. The mobile phase consists of acetonitrile (A) and ultra pure water (B). The gradient elution from 15% to 25% A in 0–1.5 min, 25% A in 1.5–3.5 min, 25–28% A in 3.5–7.0 min, 28–40% A in 7.0–10.0 min, 40–90% A in 10.0–15.0 min, 90–15% A in 15–20.0 min and there-equilibration time of gradient elution was 3 min. The flow rate was 0.3 mL/min and the injection volume was 2 μL. The PDA detector wavelength was set at 203 nm for acquiring chromatograms. Ultra-violet (UV) spectra were acquired from 200 to 400 nm. PRS prepared as mentioned above. Four reference substances were used for the qualitative analysis: polyphyllin VII, polyphyllin VI, polyphyllin II and polyphyllin I were purchased from National Institute for the Control of Drug and Biological Products (Beijing, China). The compounds were vertified based on comparing individual peak retention times with that of the reference substances.

2.3. Chemicals

CCl_4 (Sigma-Aldrich, Co., USA); RASAL1 and ERK1/2 rat anti-human monoclonal antibody (Beijing Biosynthesis Biotechnology Co., Ltd., China); Generic two step immunohistochemical kit (Beijing Zhongshan Golden bridge Biotechnology Company, Co., Ltd.); Trizol (Invitrogen Co., Ltd., USA); Reverse transcription Kit (Thermo Co., Ltd., USA); PCR Master Mix (Thermo Co., Ltd., USA); Rabbit anti p-ERK1/2 polyclonal antibody (Cell signaling, Co., Ltd., Japan).

2.4. Animal and experimental model

Forty male Sprague–Dawley (SD) rats weighing 180–200 g were purchased from Laboratory Animal Center, Medical University of Anhui Province. All the experiments were approved by national legislations of China and local guidelines. Rats were housed in a pathogen-free experimental animal room with a controlled environment (25 °C and 12 h light/dark cycle).

After one week acclimatization period, a total of 40 SD rats were randomly divided into the control group, the model group, the PRS high dose group (PRS H) and the PRS low dose group (PRS L), with 10 rats in each. The model, PRS H and PRS L group were injected intraperitoneally with 50% CCl_4 dissolved in olive oil for 16 consecutive weeks (2.0 mL/kg body weight/rat, twice a week) to induce hepatic fibrosis as according to D'Argenio et al. (2013) and Furtado et al. (2014).