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Lycium barbarum polysaccharides protect mice liver from carbon tetrachloride-induced oxidative stress and necroinflammation

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ABSTRACT

Ethnopharmacological relevance: Lycium barbarum has been used as a traditional Chinese medicine to nourish liver, kidneys and the eyes.
Aim of the study: We investigated the protective mechanisms of Wolfberry, *Lycium barbarum* polysaccharides (LBP) in carbon tetrachloride (CCl_4)-induced acute liver injury.
Materials and methods: Mice were intraperitoneally injected with a 50 μl/kg CCl_4 to induce acute hepatotoxicity (8 h) and were orally fed with LBP 2 h before the CCl_4 injection. There were six experimental groups of mice (n = 7–8 per group), namely: control mice (vehicle only; 1 mg/kg LBP or 10 mg/kg LBP), CCl_4-treated mice and CCl_4 + LBP treated mice (1 mg/kg LBP or 10 mg/kg LBP).
Results: Pre-treatment with LBP effectively reduced the hepatic necrosis and the serum ALT level induced by CCl_4 intoxication. LBP remarkably inhibited cytochrome P450 2E1 expression and restored the expression levels of antioxidant enzymes. It also decreased the level of nitric oxide metabolism and lipid peroxidation induced by CCl_4. LBP attenuated hepatic inflammation via down-regulation of proinflammatory mediators and chemokines. Furthermore, LBP promoted liver regeneration after CCl_4 treatment. The protective effects of LBP against hepatotoxicity were partly through the down-regulation of nuclear factor kappa-B activity.
Conclusion: LBP is effective in reducing necroinflammation and oxidative stress induced by a chemical toxin, thus it has a great potential use as a food supplement in the prevention of hepatic diseases.

1. Introduction

Wolfberry, also known as Goji berry, is the fruit of two close species in the family *Solanaceae*: *Lycium barbarum* and *Lycium chinense*, which has a long history of use in Chinese and Indian medicine (Chang and So, 2008). Recently, Wolfberry and its juice products are also being used as health food and anti-aging remedy in Western countries (Potterat, 2010). Modern analytical methods identified that the polysaccharides fractions (often referred to LBP, *Lycium barbarum* polysaccharides) represent the most important group of substances in Wolfberry, both quantitatively and qualitatively (Wang et al., 2009). LBP is found to be complex glycopeptides consisting of acidic heteropolysaccharides and polypeptides or proteins. It also contains six monosaccharides (arabinose, rhamnose, xylose, mannose, galactose and glucose), galacturonic acid and eighteen amino acids (Amagase and Farnsworth, 2011). In the past years, the main research focused on the therapeutic properties of LBP as an antioxidative and immunomodulatory drug against diabetes and atherosclerosis. Oral administration of LBP protected heat-induced damage in rat testes *in vivo* and H_2O_2-induced oxidative damage in mouse testicular cell *in vitro*, which demonstrated the beneficial effect of LBP in fertility (Luo et al., 2006). Besides increasing the activities of antioxidative enzymes (e.g. glutathione peroxidase, superoxide dismutase, and catalase), oral infusion of LBP also decreased the level of triglycerides, low-density lipoprotein (LDL) and total cholesterol in high-fat diet fed mice (Ming et al., 2009). The immunomodulatory and anticancer properties of LBP in rodent models (e.g. stimulation of dendritic cell immunogenicity) have also been investigated (Chen et al., 2009). In addition to these properties, the pro-apoptotic effect of LBP also contributes to its anticancer property. It has been reported that in human hepatoma cell line, administration of LBP inhibits cell proliferation with S phase arrest and promotes cell apoptosis (Zhang et al., 2005). Considering the beneficial effects of LBP, it holds great potential as food supplement in the prevention of cancers or other

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diseases related to metabolic syndrome with oxidative stress and dysfunction of the immune system as the major underlying risk factors.

Acute liver failure is a kind of hepatic disorder caused by acute severe injuries, which leads to the functional loss of 80–90% of liver cells within 1–4 weeks (O'Grady et al., 1993). Overdose of toxin ingestion, viral infection, metabolic disorder, immunological insult and ischemic injury are common causes of acute liver failure (Bernal et al., 2010). Carbon tetrachloride (CCl_4) at a high dose often rapidly causes cellular necrosis, oxidative stress and inflammation which leads to acute liver injury and failure (Weber et al., 2003). The hepatotoxicity of CCl_4 includes two steps. The first one is the production of free radicals (CCl_3* and CCl_3OO*) through metabolism of cytochrome P450 system, which induces lipid peroxidation. The second phase involves the activation of Kupffer cells, the macrophages of the liver, which is accompanied by the production of inflammatory and profibrogenic mediators. Applying natural herb or herbal derivative as protective agent against CCl_4-induced toxicity received much interest in recent years such as. epigallocatechin gallate from green tea (Chen et al., 2004) and ginsan from the root of *Panax ginseng* (Shim et al., 2010). As an antioxidant herbal medicine, the property of LBP in the prevention and protection of liver injury remains unclear. In the present study, we evaluated the protective effects of LBP in the CCl_4 induced acute liver damage with several proposed mechanistic pathways.

2. Materials and methods

2.1. Materials and reagents

Lycium barbarum polysaccharides (LBP) was purchased from Hong Kong Institute of Biotechnology (Shatin, Hong Kong) and was provided by Versitech Ltd. Neutral sugar composition analysis showed that the LBP powder contained about 35% arabinose, 16% galactose, 10% rhamnose, and small portions of glucose, xylose, mannose and carotenoids. The purity of LBP was found to be ~62% (w/w carbohydrates). Carbon tetrachloride was purchased from Tianjin Baishi Chemical (Tianjin, China). Murine anti-nitrotyrosine monoclonal antibody and rabbit anti-cytochrome P450 2E1 (CYP2E1) polyclonal antibody were purchased from Zymed (San Francisco, CA) and Millipore (Billerica, MA), respectively. Gel-shift antibodies of p50 and p65 were bought from Santa Cruz Biotechnology Inc. (Santa Cruz, CA).

2.2. Animals and treatment

Healthy 8–10 weeks old male and female C57BL/b6N mice were purchased from the Laboratory Animal Unit (LAU), The University of Hong Kong. Before CCl_4 treatment, mice were kept under standard conditions for 1 week with free access to chow and tap water. Mice were divided into six groups (n = 7–8 per group) namely: (1) control with vehicle administration (normal saline and olive oil); (2) CCl_4 treatment (50 μl/kg in olive oil; intraperitoneal injection); (3) 1 mg/kg LBP treatment, orally fed; (4) 10 mg/kg LBP treatment, orally fed; (5) CCl_4 and 1 mg/kg LBP pre-treatment; and (6) CCl_4 and 10 mg/kg LBP pre-treatment. Selection of LBP dosages was based on previous studies on neuronal protection (Chiu et al., 2010). LBP was orally fed 2 h before the CCl_4 injection. After 8 h CCl_4 treatment, mice were euthanized by overdose of anesthesia according to the protocols approved by the Committee of Animal Use for Research and Teaching at The University of Hong Kong. The laboratory animal unit of The University of Hong Kong is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC international). Mice blood and liver tissues were collected for further analyses.

2.3. Tissue and blood samples processing and histological analysis

Mice serum were collected by centrifugation of whole blood sample at 1000 × *g* for 10 min at 4 °C and stored at −80 °C. After fixation in 10% phosphate-buffered formalin for 72 h, liver tissue blocks were processed histologically, embedded in paraffin, cut to 5 μm sections, and stained with hematoxylin and eosin (H&E staining). H& E stained sections were evaluated for the presence of necrosis and inflammation.

2.4. Serum alanine aminotransferase (ALT) assay

As an enzyme indicator of hepatic injury, the hepatotoxicity of CCl_4 can be detected by the increased level of ALT in the serum. The ALT assay was performed by procedures described previously (Tipoe et al., 2010).

2.5. Determination of malondialdehyde (MDA) level

To determine the role of CCl_4 and LBP treatments on hepatic lipid peroxidation and MDA levels, liver samples were measured by using a Bioxytech LPO-586™ kit (OxisResearch, Portland, OR). The reaction product was measured spectrophotometrically at 586 nm. Standard curves were constructed using 1,1,3,3-tetraethoxypropane as the standard. The MDA levels were normalized with corresponding protein amount determined using a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA) and the values expressed were represented as percentages against the control levels.

2.6. RNA extraction and quantitative reverse-transcription polymerase chain reaction (realtime-PCR)

To study the molecular mechanism of CCl_4 and LBP treatments on the liver, total RNA was extracted from liver tissue samples by using illustra™ RNAspin mini kit (GE healthcare, UK) and then reverse-transcribed with SuperScript™ First-Strand Synthesis System (Invitrogen, Calsbad, CA).

The mRNA expression levels of tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), glutathione peroxidase (GPx), catalase (CAT), Cu/Zn SOD, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2), KC (murine IL-8 ortholog), interleukin-6 (IL-6), and transforming growth factor-beta1 (TGF-β_1) were measured by real-time PCR. The primer sequences are listed in Table 1. Parallel amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control. Relative quantification was done by using the $2^{-\Delta\Delta Ct}$ method. The relative expression of the specific gene to the internal control was obtained and then expressed as percentage of the control expression in the figures.

2.7. Western blot analysis

Protocols for cytosolic and nuclear protein extraction, including Western blotting were performed as previously described (Tipoe et al., 2007, 2010).

2.8. Enzyme-linked immunosorbent assay (ELISA) measurement

ELISA measurements of IL-1β, IL-6, KC, and TGF-β_1 protein expression levels were performed using kits from PeproTech (PeproTech Inc., Rocky Hill, NJ). ELISA of COX-2 was conducted using a kit purchased from EIAab (Wuhan EIAab Science, Wuhan, China).