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Protective effects of Ginsenoside Rg1 against carbon tetrachloride-induced liver injury in mice through suppression of inflammation

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ABSTRACT

Background: AMP-activated protein kinase (AMPK) is one of the principal cellular energy sensors participating in maintenance of energy balance but recent evidences also suggested that AMPK might be involved in the regulation of inflammation.
Study design/methods: Ginsenoside Rg1 (Rg1) was used to investigate the potential roles of AMPK in carbon tetrachloride (CCl_4)-induced hepato-toxicity. The experimental data indicated that treatment with Rg1 significantly decreased the elevation of plasma aminotransferases and alleviated hepatic histological abnormalities in CCl_4-exposed mice. Treatment with Rg1 also inhibited the increase of myeloperoxidase (MPO) and malondialdehyde (MDA), the induction of TNF-α, IL-6, inducible nitric oxide synthase (iNOS), nitric oxide and the upregulation of matrix metalloproteinase 2 (MMP-2), MMP-3 and MMP-9 in mice exposed to CCl_4. These effects were associated with suppressed nuclear accumulation of NF-κB p65.
Conclusion: These results indicated that Rg1 effectively suppressed the inflammatory responses and alleviated liver damage induced by CCl_4, implying that AMPK activation might be beneficial for ameliorating inflammation-based liver damage.

Introduction

Liver disease has been confirmed as one of the most serious health problems in the world (Williams 2006), which can be caused by many factors including hepatitis virus infection, induction of drugs and toxins. Carbon tetrachloride (CCl_4) is a well-known environmental biohazard, which can cause particularly toxic to the liver. Experimental and clinical studies increasingly show that CCl_4 induced hepatic injury, a classic experimental model, has been extensively used to evaluate the potential of drugs and dietary antioxidants against the oxidative damage (Basu 2003). Free radicals, such as trichloromethyl (CCl_3 and/or CCl_3OO) and oxygen-centered lipid radicals (LO and/or LOO), are pivotal in CCl_4-induced hepatotoxicity, which are generated during CCl_4 metabolism by hepatic cellular cytochrome P450 (Recknagel et al. 1989; Li et al. 2010). In addition, the activation of Kupffer cells also contributes to the liver injury through releasing both direct toxic products and cytokines which promote inflammatory response (Taniguchi et al. 2004). The liver injury may be prevented or treated by blocking or retarding the process of oxidative stress and inflammation (Lin et al. 2012).

Recently, the interplay between energy metabolism pathway and inflammatory response was highly concerned (Delmastro-Greenwood and Piganelli 2013; McGettrick and O'Neill 2013). Several pathways contribute to CCl4-induced inflammatory response and one of the central pathways is through the induction of AMPK, a conserved cellular energy status sensor. The cellular energy status is monitored by various energy sensors such as AMP-activated protein kinase (AMPK). AMPK is activated by the increased level of adenosine monophosphate (AMP) under falling

Abbreviations: AMPK, AMP-activated protein kinase; Rg1, Ginsenoside Rg1; MPO, myeloperoxidase; MDA, malondialdehyde; NF-κB, Nuclear factor-kappaB; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; iNOS, nitric oxide synthase; MMP-2, metalloproteinase 2.

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energy status (Hardie et al. 2012). The activated AMPK helps the cells to restore energy homoeostasis via activating the catabolic pathways that generate ATP while deactivating the anabolic pathways that consume ATP (Dunlop and Tee 2013). AMPK is also a serine–threonine kinase that can phosphorylate and subsequently inactivate sirtuin 1 (Sirt1), thereby attenuating steatosis. Expression of the Sirt1, nicotinamide adenine dinucleotide-dependent class III histone deacetylase, is decreased in mice treated with CCL4, resulting in increased levels of several inflammatory cytokines. In addition, recent studies have found that the induction of pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) by lipopolysaccharide (LPS) could be suppressed by over-expression of constitutively active AMPK but could be enhanced by over-expression of dominant-negative AMPK or short hairpin RNA targeting AMPK (Yang et al. 2010). Simultaneously, MAPK may act upstream of NF-κB signaling because the inhibitors of MAPK activation have a negative effect on NF-κB activation. NF-κB activation is largely involved in the gene expression of proinflammatory cytokines and chemokines and is responsible for the expression and activity of inflammatory factor. Therefore, the inhibition of downstream NF-κB signaling may prevent proinflammatory events. These emerging evidences suggested that AMPK-Sirt1-NF-κB might be involved in the regulation of inflammation, an energy-intensive pathological response (Pearce et al. 2013).

Nowadays, many hepatoprotective medicines have been widely used, and however, some of them have potential adverse effects (Tian et al. 2012). Natural products from medicinal plants, especially to traditional herbal medicine, have been attracted much attentions as effective and safe alternative treatments for liver diseases (Ma et al. 2009). *Panax ginseng* C. A. Mey is widely used as a traditional herbal medicine and exhibits many functional activities such as antioxidant, anti-inflammatory, and anti-aging potencies. Ginsenosides, Rg1 is one of the most active and abundant steroid saponin that shares structural similarity with many steroid hormones. The most importantly is that extract of ginkgo biloba injection, which is already available to treat disturbance of blood circulation as prescription drug. Rg1 has been found as an antioxidant substance and attenuated the oxidative damage in liver of thioacetamide treated rats (Deng and Zhang 1991; Geng et al. 2010). In the light of all these bases, the present study was to investigate the hepatoprotective effect of ginsenoside-Rg1 on CCl_4-induced hepatotoxicity in mice and then to explore the possible mechanisms of the action.

Materials and methods

Chemicals and materials

Rg1 (purities $>$ 98%) were purchased from National Institutes of Food and Drug Control of China (Beijing, China). CCl_4 was purchased from Kaixing Chemical Industry Co., Ltd. (Tianjin, China). Silymarin was obtained from Sigma Chemical Company (Milan, Italy). The detection kits including alanine transaminase (ALT), aspartate transaminase (AST), myeloperoxidase (MPO), malondialdehyde (MDA), superoxidase dismutase (SOD) and nitric oxide (NO) were all purchased from Nan jing Jiancheng Institute of Biotechnology (Nanjing, China). The enzyme-linked immunosorbent assay (ELISA) kits for determination of IL-6, TNF-α and the nuclear and cytoplasmic protein extraction kit were produced by Nanjing KeyGEN Biotech. CO., Ltd. (Nanjing, China). The rabbit anti mouse nuclear factor kappa B (NF-κB) p65 was purchased from Cell Signaling Technology (Beverly, MA, USA). Horseradish peroxidase-conjugated goat anti-rabbit and goat mouse antibodies were purchased from GE Healthcare (London, UK). PVDF membranes were purchased from Roche (Basel, Switzerland). All other chemicals were of reagent grade.

Animals

50 male Kunming mice (18–22 g) were purchased from the Experimental Animal Center of China Pharmaceutical University (Nanjing, China). The mice were used after one week of acclimatization. They were kept in departmental animal house in well cross ventilated room at 24 °C, relative humidity (45–55%), and a light–dark cycle of 12 h during the experiments. All mice were housed uniformly per cage (free access to tap water and diet) until the end of the study. All experimental procedures were approved by the Animal Care and Use Committee of China Pharmaceutical University, and performed in strict accordance with the PR China Legislation Regarding the Use and Care of Laboratory Animals.

Experimental design

After environmental adaptation, the mice were randomly allocated in to normal group, model group, silymarin-treated group and Rg1-treated groups. Each group contained 10 animals. The mice were received 0.5% CMC-Na distilled water solutions (p.o.) in normal and model groups, Rg1 (20, 40 mg/kg dissolved in 0.5 % CMC-Na) in Rg1 treated groups, and the positive drug (20 mg/kg) in silymarin treated group. All administrations were conducted for 7 consecutive days. On the 8th day, all mice except those in normal group was injected intraperitoneally with 0.3% CCl_4 (10 ml/kg, dissolved in olive oil), whereas the animals in normal group were received olive oil alone (i.p.). Twenty-four hours after injection, the mice were sacrificed under ether anesthesia. The blood was collected and serum was separated immediately. The fresh liver tissues were excised, blotted, weighed and stored at −80 °C for further experiments.

Determination of liver enzymes

The levels of ALT, AST in liver and serum were assayed based on the manufacturer's instruction of the kits. The value of each sample was calculated according to the standard curve.

Histological investigation

Paraffin embedded sections of the livers were cut with 5 μm *thicknes sand* examined after staining with H&E using alight microscope (Nikon Eclipse TE2000-U, NIKON, Japan) and then photographed at 200× magnification.

Determination of cytokines by ELISA

The protein levels of TNF-α and IL-6 in plasma were determined using ELISA kits according to the manufacturer's instructions (NeoBioscience, China).

Determination of NO

The levels of NO in plasma were determined with NO assay kit according to the manufacturer's instructions (Beyotime, China). The values of NO were assessed according to the absorbance measured at 540 nm.

Determination of MPO, SOD and MDA

The frozen liver tissues were homogenized in phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide. The activities of MPO, SOD and MDA were determined with MPO, SOD and MDA assay kit according to the manufacturer's instructions (Nanjing Jiancheng, China).