International Immunopharmacology 47 (2017) 126–133

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Inhibitory effects of quercetin on the progression of liver fibrosis through the regulation of NF-κB/IκBα, p38 MAPK, and Bcl-2/Bax signaling

Rong Wang [a,1], Hai Zhang [b,1], Yuanyuan Wang [a], Fuxing Song [a], Yongfang Yuan [a,*]

[a] Department of Pharmacy, Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine, 280 Mo He Rd, Shanghai 201999, China
[b] Department of Pharmacy, Shanghai First Maternity and Infant Hospital, Tong Ji University School of Medicine, 536 Changle Road, Shanghai 200080, China

ARTICLE INFO

Article history:
Received 11 November 2016
Received in revised form 22 March 2017
Accepted 29 March 2017
Available online 6 April 2017

Keywords:
Liver fibrosis
Quercetin
NF-κB
p38 MAPK, apoptosis pathway

ABSTRACT

Quercetin, a natural flavonoid, has been used as a nutritional supplement for its anti-inflammatory and antioxidative properties. Quercetin was reported to exhibit a wide range of pharmacological properties, including its effect on anti-hepatic fibrosis. However, the anti-fibrotic mechanisms of quercetin have not been well-characterized to date. This study aimed to investigate the protective effects of quercetin on carbon tetrachloride (CCl_4)-induced liver fibrosis in rats and to clarify its anti-hepatofibrotic mechanisms. We demonstrated that quercetin exhibited in-vivo hepatoprotective and anti-fibrogenic effects against CCl_4-induced liver injury by improving the pathological manifestations, thereby reducing the activities of serum total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and decreasing the serum levels of hyaluronic acid (HA), laminin (LN), type IV collagen (IV-C) and procollagen III peptide (PIIIP). Furthermore, treatment with quercetin 5–15 mg/kg inhibited the activation of NF-κB in a dose-dependent manner via inhibition of IκBα degradation and decreased the expression of p38 MAPK by inhibiting its phosphorylation. Additionally, in a dose-dependent manner, quercetin down-regulated Bax, up-regulated Bcl-2, and subsequently inhibited caspase-3 activation. Moreover, quercetin regulated inflammation factors and hepatic stellate cells (HSCs)-activation markers, such as TNF-α, IL-6, IL-1β, Cox-2, TGF-β, α-SMA, Colla1, Colla2, TIMP-1, MMP-1, and desmin. Taken together, quercetin prevented the progression of liver fibrosis in SD rats. The anti-fibrotic mechanisms of quercetin might be associated with its ability to regulate NF-κB/IκBα, p38 MAPK anti-inflammation signaling pathways to inhibit inflammation, and regulate Bcl-2/Bax anti-apoptosis signaling pathway to prevent liver cell apoptosis.

1. Introduction

Liver fibrosis is a wound healing response to chronic liver injury and a potential contributor to liver failure, cirrhosis and cancer. This progressive pathological process is resulted from the accumulation of extracellular matrix proteins, in which hepatic stellate cells (HSCs) play an important role in the deposition of scar formation and fibrous tissue [1,2]. However, there are few therapeutic drugs or strategies for the patients with liver fibrosis up to now. In recent years, Chinese herbal medicine attracted more and more attentions for the anti-fibrotic treatment, which have many advantages, such as low toxicity and fewer side effects [3–5].

Quercetin (chemical structure is shown in Supplement Fig. 1) is a polyphenolic flavonoid, which contains a number of phenolic hydroxyl groups that have strong anti-inflammatory and antioxidant properties [6]. Growing experimental data have illustrated that quercetin has the ability to offer hepato-protection and inhibit liver fibrosis, however, the exact molecular mechanisms still remain unclear [7–9].

NF-κB, as a transcriptional regulator, plays an important role in the development of liver fibrosis as follows: 1) NF-κB regulates hepatocyte injury, the primary trigger of fibrogenic responses in the liver; 2) NF-κB regulates multiple essential functions in HSCs; and 3) NF-κB regulates inflammatory signals elicited in macrophages and other inflammatory cells in the liver, and at the same time promotes the expression of inflammatory factors, such as COX-2, TNF-α, and IL-6 [10,11]. Many studies demonstrated that inhibition of activated NF-κB may prove to be a useful strategy for promoting regression of liver fibrosis [12–14]. Therefore, it was essential to find effective drugs that exert a moderate effect on NF-κB activity to avoid the increase in liver fibrosis. Interestingly, quercetin was found to inhibit a broad spectrum of kinases, down-regulate NF-κB, and as a consequence, decrease pro-inflammatory cytokine expression [6,15]. Additionally, it had been reported that quercetin prevents oxidative stress and NF-κB activation in gastric mucosa of portal hypertensive rats. Furthermore, quercetin had been found to modulate ochratoxin A-induced oxidative stress and redox signaling in HepG2 cells through down-regulation of NF-κB [16,17].

* Corresponding author.
E-mail address: nmxyyf@126.com (Y. Yuan).
[1] The first two authors equally contributed to this work.

http://dx.doi.org/10.1016/j.intimp.2017.03.029
1567-5769/

The MAPK family can serve as focal points in response to cell proliferation, differentiation, and regulation of specific metabolic pathways [18]. An essential member of the MAPK super family is p38 MAPK, which plays a role in the response of HSCs to liver injury and inflammation [19]. Inhibition of p38 MAPK phosphorylation can limit the activation of HSCs, leading to inhibition of the development of liver fibrosis [20]. Many reports have shown that quercetin can inhibit the p38 MAPK signaling pathway. Therefore, using quercetin to target p38 MAPK or NF-κB signaling pathway may be a treatment strategy to inhibit fibrosis.

Moreover, liver cell apoptosis promotes liver fibrosis, whereas activated HSCs apoptosis limits liver fibrosis. The Bcl-2 family, Bcl-2 and Bax, play pivotal roles in apoptosis by regulating mitochondrial outer membrane permeability [21]. The relative ratio of Bcl-2/Bax may serve as a key sensory switch, dictating the initiation of cell apoptosis [21–22]. Inhibiting liver cell apoptosis may potentially attenuate liver fibrosis by permitting accumulation of activated HSCs [23]. Therefore, regulating Bcl-2/Bax signaling may block hepatocyte apoptosis to attenuate liver injury, which may be the anti-fibrosis strategy of quercetin.

In summary, regulation of NF-κB/IκBα, p38 MAPK, and Bcl-2/Bax signaling pathways was the potential therapeutic strategy in the treatment of liver fibrosis. Accordingly, it was speculated that therapeutic effect of quercetin on liver fibrosis could be mediated through suppressing NF-κB and p38 MAPK signaling pathways, inhibiting inflammatory response, and preventing liver cell apoptosis. Therefore, this study aimed to investigate the protective effects of quercetin on carbon tetrachloride (CCl_4)-induced liver fibrosis in rats and to clarify its anti-hepatofibrotic mechanisms.

2. Materials and methods

2.1. Materials

We used following materials in our study: Quercetin (Shanghai Nature Standard R&D and Biotech Co., Ltd., Shanghai, China, purity 98.3%); CCl_4 (Shanghai Jinghua Scientific & Technological Research Institute, Shanghai, China); NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, Waltham, MA, USA, Product No 78833, Lot#PG201976A); monoclonal anti-p38 MAPK, anti-NF-κBp65, anti-p-p38 MAPK, and anti-IκBα rabbit antibodies (Santa Cruz Biotechnology Inc., Dallas, TX, USA); monoclonal anti-Bcl-2, anti-caspase-3anti-Bax, β-tubulin, and histone H3 rabbit antibodies (Cell Signaling Technology, Danvers, MA, USA); the horseradish peroxidase (HRP)-labeled GAPDH antibody (Bioworld Technology Inc., St Louis Park, MN, USA); the secondary antibody, HRP-labeled goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA); phenylmethanesulfonyl fluoride, the radioimmunoprecipitation assay lysis buffer, and sodium dodecyl sulfate -polyacrylamide gel electrophoresis protein-loading buffer (5×) (Beyotime Biotechnology Corporation, Shanghai, China); TRIzol®reagent (Thermo Fisher Scientific); and the rabbit IgG immunohistochemistry (streptavidin-biotin complex) and diaminobenzidine colorimetric kits (BOSTER Biological Technology Ltd., Wuhan, China). The Premix Ex Taq ™Hot Start Version reagent (Cat #RR030A) Prime-Script™RT Master Mix reagent (Cat #RR036A),and SYBR®Premix Ex Taq™II (Tli RNaseH Plus) reagent (Cat #RR820A) were prepared by TaKaRa Co., Ltd., Kyoto, Japan.

2.2. Animal models

Forty-eight SD rats (6 weeks; 180–220 g) were purchased from the B&K Universal Group Ltd. (Shanghai, China) and bred in an experimental animal unit of Shanghai 9th People's Hospital. All the rats were housed in polypropylene cages under a 12 h light-dark cycle at 25 °C and humidity of 60–70%. The rats had free access to a pellet diet and were allowed two weeks to acclimate to their surroundings after arrival.

Rats were divided randomly into four groups as follows: normal group (n = 12); model group (n = 12); quercetin 5 mg/kg group (n = 12); quercetin 15 mg/kg group (n = 12). Rats in model, quercetin 5 mg/kg and 15 mg/kg groups received CCl_4 orally (1:1 in olive oil) 1 mL/kg twice a week for 8 weeks. During the 8 weeks, rats in quercetin 5 mg/kg or 15 mg/kg groups were intraperitoneally injected with an additional quercetin 5 mg/kg or 15 mg/kg once every day. After 8 weeks, surviving rats were weighed and anesthetized with pentobarbital sodium (Shanghai Beizhuo Biochemical & Technological Co., Ltd., Shanghai, China) administered via the intraperitoneal route. Blood was taken from the abdominal aorta and centrifuged (1000 ×*g* for 15 min) to obtain serum, which was then stored at −80 °C. The same part of each liver was fixed in 10% neutral formalin, and then stored at −80 °C until further use.

The study protocol was approved by the Animal Ethics Committee of North Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine (Shanghai, China). The study protocol complied with regulations regarding animal use set by the National Science and Technology Commission of China (Beijing, China).

2.3. Calculation of the liver index

The liver index was calculated as the following formula: Liver index = (liver weight / body weight) × 100.

2.4. Determination of parameters of liver disease in the blood

Serum levels of total bilirubin (TBIL), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured by enzyme-coupled oxidation of the reduced form of nicotinamide adenine dinucleotide (NADH) to the oxidized form of nicotinamide adenine dinucleotide (NAD+) using an automatic biochemical analytic instrument (Beckman LX-2, CA, USA). According to manufacturer's operating instructions, a certain volume of sample and reagent was added to the test cup in the analyzer, and their activity were manifested by the rate of oxidation based on reflectance spectrophotometry.

Concentrations of HA, IV-C, LN and PIIIP were measured by radioimmunoassay using commercial kits (Beifang Institute of Biotechnology, Beijing, China). Samples, standard samples, reagents and antibodies were added to the corresponding tubes according to manufacturer instructions. After mixing, tubes were incubated for 4 h at 4 °C, and then the second antibody was added. Subsequently, the tubes were centrifuged at 1500 ×*g* for 15 min, and supernatants were removed. Radioactivity levels were measured and binding rates calculated according to manufacturer instructions.

2.5. Histopathological and immunohistochemical examination

2.5.1. Histopathological examination

Liver samples were fixed with 10% formaldehyde. Tissue pieces were dehydrated in alcohol and embedded in paraffin. Sections were mounted on glass slides. All histological examinations were undertaken by a very experienced pathologist blinded to the study protocol. Microscopic fields in all liver sections were randomly selected for examination using a light microscope (Ti-S Inverted fluorescence microscope, Type 108, Nikon Corporation, Tokyo, Japan). The criteria used for scoring fibrosis degree were: 0, no obvious fibrosis; 1, fibrosis present (collagen fibers that extend from the portal triad or central vein to peripheral regions); 2, mild fibrosis (few collagen fibers extending without formation of compartments); 3, moderate fibrosis (collagen fibers with formation of "pseudo leaves"); 4, severe fibrosis (many collagen fibers with thickening of partial compartments and formation of "pseudo lobes"). The collagen deposition were evaluated by staining with Masson's trichrome, and the quantitative assays were performed using the image software according to the instructions.