Quercetin protects liver injury induced by bile duct ligation via attenuation of Rac1 and NADPH oxidase1 expression in rats

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BACKGROUND: Bile duct ligation (BDL) and subsequent cholestasis are correlated with oxidative stress, hepatocellular injury and fibrosis. Quercetin is a flavonoid with antifibrotic, and hepatoprotective properties. However, the molecular mechanism underlying quercetin-mediated hepatoprotection is not fully understood. The current study was to evaluate mechanisms of hepatoprotective effect of quercetin in BDL rat model.

METHODS: We divided male Wistar rats into 4 groups (n=8 for each): sham, sham+quercetin (30 mg/kg per day), BDL, and BDL+quercetin (30 mg/kg per day). Four weeks later, the rats were sacrificed, the blood was collected for liver enzyme measurements and liver for the measurement of Rac1, Rac1-GTP and NOX1 mRNA and protein levels by quantitative PCR and Western blotting, respectively.

RESULTS: Quercetin significantly alleviated liver injury in BDL rats as evidenced by histology and reduced liver enzymes. Furthermore, the mRNA and protein expression of Rac1, Rac1-GTP and NOX1 were significantly increased in BDL rats compared with those in the sham group (P<0.05); quercetin treatment reversed these variables back toward normal (P<0.05). Another interesting finding was that the antioxidant markers e.g. superoxide dismutase and catalase were elevated in quercetin-treated BDL rats compared to BDL rats (P<0.05).

CONCLUSION: Quercetin demonstrated hepatoprotective activity against BDL-induced liver injury through increasing antioxidant capacity of the liver tissue, while preventing the production of Rac1, Rac1-GTP and NOX1 proteins.

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KEY WORDS: quercetin; Rac1; NOX1; liver fibrosis; oxidative stress

Introduction

Liver fibrosis is characterized by the excessive production of extracellular matrix proteins (collagen) that occurs in most types of liver injury. Advanced liver fibrosis leads to cirrhosis, liver failure, and hepatocellular carcinoma (HCC).[1, 2] Cholestasis is a clinically substantial event that contributes to liver fibrosis. Cholestatic liver fibrosis is identified by excessive collagen production and deposition, which is mediated by reactive oxygen species (ROS). Continuous cholestasis leads to damage of hepatocytes and subsequent liver fibrosis, cirrhosis and death.[3]

Cellular oxidative damage advances when the balance between ROS-generating systems and ROS scavenging ones tilts in favor of the former.[4-6] Among all the antioxidants that are available in the body, thiols constitute the major portion which plays a significant role in defense against ROS.[7, 8] Carbonylation of proteins and malondialdehyde (MDA) are two important oxidative stress markers.[9] Carbonylation of proteins is an indicator of severe oxidative damage and disease-derived protein dysfunction that can be promoted by ROS.[4] MDA is the most abundant aldehyde produced during lipid peroxidation, and its measurement is indicative of oxidative stress.[10]

Nicotinamide adenine dinucleotide phosphate oxidase (NOX) is a major intracellular producer of ROS. NOX

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is an enzyme system that induces the reduction of molecular oxygen to superoxide and plays a key role in the pathogenesis of liver fibrosis.[11] The NOX family consists of seven different members (NOX1-5 and the dual oxidases Duox1 and 2). Among the NOX family, NOX1, NOX2 and NOX4 are expressed on hepatic stellate cells (HSCs) and may contribute to liver fibrosis. One integral component in the activation of NOX is Ras-related C3 botulinum toxin substrate 1 (Rac1).[12, 13] Rac1 belongs to a subfamily of small GTP-binding proteins, and regulates many cellular functions including proliferation, gene expression, inflammation, apoptosis and tumor progression.[14, 15] Upon Rac1-GTP (biologically active form of Rac1) translocation to the membrane-bound cytochrome complex, enzymatically active NOX1 and NOX2 are released. Increased HSC-NOX activity through Rac1 induces liver fibrogenesis.[12]

Use of herbal drugs in the treatment of liver diseases has a long tradition. Flavonoids are plant-derived antioxidants with major suppressive effects on liver fibrosis through their antioxidant, anti-fibrotic and anti-carcinogenic properties.[16] The most abundant flavonoid in nature, quercetin presents in large amounts in vegetables, fruits, tea and olive oil. Recent evidence has demonstrated the therapeutic effects of quercetin against cholestasis liver injury.[17-19] Its therapeutic characteristics have been attributed to its phenolic hydroxyl groups.[17, 20]

Bile duct ligation (BDL) is the most common model used to induce obstructive cholestatic damage in mice and rats. BDL models can give valuable information about cholestasis and subsequent liver fibrosis.[17] Based on evidence that Rac1 and NOX1 expressions are associated with oxidative stress and liver fibrogenesis, we investigated the impact of quercetin on Rac1, Rac1-GTP, and NOX1 expression in BDL rat model. As the indices of oxidative stress, we also quantified protein carbonylation and total reduced thiols, and also assessed enzymatic activities of superoxide dismutase (SOD) and catalase.

Methods

Animals and experimental procedures

Adult male Wistar rats (200-250 g, Pasteur Institute, Tehran, Iran) were used in this research. Rats were kept in an air-conditioned room at 25 ℃ with a 12-hour darkness/light cycle, and had free access to rat food diet and drinking water. All of the study's protocols agree with the current ethical considerations of local ethical committee of animal use. The 32 rats were randomly divided into 4 groups: sham, sham+quercetin (30 mg/kg per day), BDL and BDL+quercetin (30 mg/kg per day). Quercetin was suspended in 5% CMC (Sigma Chemicals Co., USA). Quercetin or the same volume/weight of the 5% CMC vehicle was gavaged once a day from the day after surgery for 28 days.[21] BDL procedure was performed as described previously.[22] Briefly, under general anesthesia, the common bile duct was exposed by a midline abdominal incision under sterile conditions. It was then ligated in two places with a silk thread and sectioned between the ligatures.[23] At the end of the 4-week period, blood samples were collected by puncturing the heart under deep anesthesia and they were centrifuged at 3000 g for 15 minutes. The serum was separated and kept at -70 ℃ until next experiments. Liver tissues were divided into three parts, one part frozen in liquid nitrogen for RNA extraction, the second part was kept at -70 ℃ to make a homogenized tissue for assessment of antioxidant parameters such as Rac1, Rac1-GTP, NOX1 and Western blotting analyses, and the last part was fixed with 10% neutral formalin for histology.

Histopathological evaluation

The liver specimens were fixed in 10% neutral formalin individually, dehydrated in alcohol and embedded in paraffin and then sections were stained with hematoxylin and eosin (HE). Lobular and portal inflammation, focal hepatocyte necrosis, ductular proliferation, and portal and septal fibrosis were investigated by a pathologist. The rats in the BDL group with no significant histopathological findings were excluded from further analysis.

Blood chemistry

The collected serum samples were examined for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) as indicators of the liver injury by using standard animal diagnostic kits (Pars Azmon Diagnostic Co., Iran) and a Roche BT3000 Auto Analyzer.

Determination of hydroxyproline level in liver tissue

An automated procedure for quantitative assay of hydroxyproline in tissue is based on the oxidation of hydroxyproline by chloramine T in aqueous solution. The oxidation product reacts with Ehrlich's reagent, and the obtained chromogen is registered in a recorder connected to the colorimeter. The amount of hydroxyproline is expressed as μg/mg tissue.[24]

Determination of MDA in tissue

The levels of MDA were determined spectrophotometrically by measuring thiobarbituric acid-reactive substances.[25] One hundred μL homogenized liver tissue supernatant were incubated with 15% trichloroacetic acid, 0.375% thiobarbituric acid, 0.25 mol/L HCl and 6.8