Chemico-Biological Interactions 269 (2017) 1–8

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

The involvement of sirtuin 1 and heme oxygenase 1 in the hepatoprotective effects of quercetin against carbon tetrachloride-induced sub-chronic liver toxicity in rats

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ARTICLE INFO

Article history:
Received 20 January 2017
Received in revised form
9 March 2017
Accepted 23 March 2017
Available online 25 March 2017

Keywords:
Quercetin
Hepatoprotection
Heme oxygenase 1
Sirtuin 1
Carbon tetrachloride
Hepatotoxicity

ABSTRACT

The present study was designed to evaluate the therapeutic potential of quercetin in a sub-chronic model of hepatotoxicity. The roles of putative antioxidant enzymes, sirtuin 1 (SIRT1) and heme oxygenase 1 (HO-1), in hepatoprotection were also addressed. Sub-chronic liver injury was induced in rats by intraperitoneal administration of 0.5 ml/kg carbon tetrachloride (CTC), once every 3 days, for 2 weeks. Some CTC rats were concurrently treated with 100 mg/kg quercetin, intragastrically, once every day, for 2 weeks. The effects of these drugs in the liver were evaluated by biochemical, histological, immunohistochemical and molecular biological studies. CTC triggered oxidative damage to the liver as unanimously shown by altered biochemical parameters and liver morphology. Furthermore, CTC highly upregulated HO-1 and SIRT1 expression levels. Concomitant treatment of rats with quercetin downregulated SIRT1 expression and ameliorated the hepatotoxic effects of CTC. However, quercetin did not have any significant effect on HO-1 expression and bilirubin levels. Collectively, these results suggest that the antioxidant and cytoprotective effects of quercetin in CTC treated rats were SIRT1 mediated and less dependent on HO-1. Thus, pharmacologic modulation of SIRT1 could provide a logic therapeutic approach in sub-chronic hepatotoxicity.

1. Introduction

Liver diseases are increasingly becoming a public health burden [1]. According to the world health organization, liver cancer and cirrhosis account for up to 175,000 deaths per year in Europe [2]. In the United Kingdom, hepatic disorders are the fifth commonest cause of death [3]. Paracetamol-induced hepatotoxicity alone, accounts for as many as 90,000 hospital visits per annum [4]. However, despite significant advances in modern medicine, hepatoprotective drugs are still inadequate. The available few compounds, such as N-acetylcysteine, have very narrow spectra and often doubtful efficacies [5]. It is therefore imperative to invest more effort in search of alternative drugs.

Among experimental drugs, natural polyphenols exhibit hepatoprotective effects through elusive mechanisms. It is widely accepted that their health benefits are sirtuin 1 (SIRT1) mediated. As a histone deacetylase, SIRT1 can alter the acetylation-deacetylation states of many regulatory proteins and act as an on-off transcription switch [6]. Furthermore, the NAD + dependency of SIRT1 makes it an important intracellular redox sensor and a critical stress-responsive gene [7], alongside well-known antioxidants such as heme oxygenase 1 (HO-1) [8]. However, the legitimacy of SIRT1 modulation by polyphenols is very controversial. How polyphenols interact with SIRT1 is unclear. Whether polyphenols directly activate SIRT1 [9], regulate SIRT1 through other molecules or act independent of SIRT1 is still open for debate [10]. This controversy is further complicated by the fact that polyphenols are numerous and structurally diverse [11]. Generalizing their effects may be misleading. This warrants further research to confirm these presumed effects and to identify other

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http://dx.doi.org/10.1016/j.cbi.2017.03.014
0009-2797/

possible druggable targets.

Quercetin is the most abundant plant flavonoid in food, with an estimated mean daily intake of about 10 mg [12]. Interest in quercetin as a potential hepatoprotective agent has been gaining momentum due to its purported anti-inflammatory, antioxidant, anti-microbial and anti-carcinogenic properties [13]. Epidemiologically, there is no substantial evidence that consumption of food rich in quercetin is hepatoprotective. This may, in part, be due to its high hydrophobicity, limited gut absorption and poor oral bioavailability [14]. However, numerous animal studies have consistently shown quercetin to be effective in mitigating many kinds of liver insults [15].

The current study was undertaken to extend our previous findings on the therapeutic potential of natural polyphenols (silymarin [16], quercetin [17], resveratrol [18]) in various experimental models of acute hepatotoxicity [19]. The study was designed to mimic sub-chronic xenobiotic-induced liver injury using carbon tetrachloride (CTC) as an inciting agent. CTC, a free radical-producing haloalkane, was chosen because of its high specificity for the liver. Effects of repeated quercetin treatment in CTC-induced hepatotoxicity were investigated. How CTC and quercetin affect SIRT1 and HO-1 expression levels was also evaluated.

2. Materials and methods

2.1. Chemicals

Carbon tetrachloride (anhydrous, ≥99.5%), quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one with HPLC purity of >95%), olive oil, anti-mouse IgG (whole molecule)-peroxidase antibody and mouse monoclonal anti-β-Actin antibody were purchased from Sigma-Aldrich (Prague, Czech Republic). Stabilized Peroxidase conjugated goat anti-Mouse and Heme oxygenase 1 antibodies were purchased from Thermo Fisher Scientific (Prague, Czech Republic). SirT1 (1F3) mouse mAb antibody was from Cell Signaling Technology through Biotech A.S. (Prague, Czech Republic). EnVision + System − HRP (Mouse) and Liquid DAB+ were purchased from DAKO (Glostrup, Denmark).

2.2. Animals

Twenty-four male Wistar rats (Velaz-Lysolaje, Czech Republic), with a weight of 290–365 g during drug administration, were used throughout the study. They were maintained under standard conditions (12-h light-dark cycle, 22 ± 2 °C temperature and 50 ± 10% relative humidity) and fed on water and standard granulated diet ad libitum. All experiments were performed in accordance with the Guiding Principles in the Use of Animals in Charles University, 1st Faculty of Medicine, and every effort was made to minimize animal suffering.

Table 1
Experimental design.

Group	Treatment
CO	DMSO + Olive oil
Q	100 mg/kg Quercetin (dissolved in DMSO) + Olive oil
CTC	DMSO + Carbon tetrachloride (mixed with Olive oil 1:1 vv)
Q + CTC	100 mg/kg Quercetin (dissolved in DMSO) + Carbon tetrachloride (mixed with Olive oil 1:1 vv)

Olive oil or carbon tetrachloride (mixed with olive oil 1:1 vv) were administered intraperitoneally, once every 3 days (days 1, 4, 7, 10, 13). DMSO or quercetin (dissolved in DMSO) were administered intragastrically (by oral gavage), 30 min after olive oil/carbon tetrachloride treatment, once every day for 14 consecutive days. All drugs were administered in a volume of 1 ml/kg body weight.

2.3. Experimental design

The rats were randomly assigned into four treatment groups (Table 1). 0.5 ml/kg CTC was administered intraperitoneally, once every 3 days, for 14 days. Some CTC rats were concurrently treated with 100 mg/kg quercetin, per os, once every day, for 14 days. These doses were selected based on previous experimental studies [20–23].

Twenty-four hours after the last treatment (day 15), the rats were anesthetized (with diethyl ether), euthanized (by exsanguination) and their blood and liver samples were collected for further analysis.

2.4. Biochemical investigations

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and direct bilirubin were measured using customized diagnostic kits per the manufacturer's instructions (Analyticon, Lichtenfels, Germany).

Nitric oxide production was determined by measuring its oxidation product, nitrite, in plasma using Griess reagent (2.5% phosphoric acid, 1% sulfanilamide, 0.1% naphthylethylenediamine). Absorbance of the mixture was detected at 540 nm. Nitrite levels were extrapolated from the sodium nitrite standard curve.

Catalase was measured spectrophotometrically in plasma based on the reaction between ammonium molybdate and hydrogen peroxide as previously reported [24].

The extent of lipid peroxidation was detected by measuring Thiobarbituric Acid Reactive Substances (TBARS) and conjugated dienes in the liver homogenate as previously described [25]. Results were expressed as concentration of these markers per unit mass of total protein (nmol/mg).

2.5. Histological evaluation

Liver samples were cut into small cubes (1 cm^3), fixed in 4% formaldehyde solution in phosphate buffered saline (PBS) and later embedded in paraffin. Then, they were sliced into 5 μm thick sections using the microtome and stained with hematoxylin and eosin for microscopic evaluation as previously described [26]. The sections (1 sample per animal) were thoroughly analyzed by the blinded observer and representative images were obtained using a Leica DMLB microscope (Leica Microsystems GmbH, Wetzlar, Germany) with Camera MC170 HD.

2.6. Immunohistochemistry

An immunoperoxidase detection was also performed on the paraffin sections. After antigen retrieval using 10 mM sodium citrate buffer (pH = 6) for 10 min in the microwave oven, the endogenous peroxidase activity and the non-specific antibody binding sites were blocked with 5% goat serum in PBS. Next, the sections were incubated with a primary antibody, mouse anti HO-1 (diluted 1:100 in PBS+1.5% goat serum), overnight at 4 °C. Visualization of antibody binding was performed using EnVision + System − HRP (Mouse) and Liquid DAB + as a substrate. Negative controls were used for all experiments. The nuclei were counterstained with hematoxylin.

2.7. Immunoblotting

Liver samples were lysed and homogenized in NP40 buffer. After centrifugation (for 15 min at 12000 rpm and 4 °C), protein concentration of the supernatant was determined (using BIO-RAD DC assay kit). Then, 40 μg of protein was separated on an SDS-PAGE gel