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Quercetin reduces serum homocysteine level in rats fed a methionine-enriched diet

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ABSTRACT

Objective: The aim of this study was to determine the effects of quercetin on homocysteine (Hcy) metabolism and hepatic antioxidant status in high methionine (Met)-fed rats.
Methods: Rats were fed for 6 wk the following diets: control, 1.0% Met, 1.0% Met and 0.1% quercetin, 1.0% Met and 0.5% quercetin, 1.0% Met and 2.5% quercetin-supplemented diets. Serum Hcy, Met, cysteine, serine, taurine, glutathione (GSH), quercetin and its metabolites, and activities of alanine transaminase (ALT) and aspartate transaminase (AST) were assayed. Hepatic malondialdehyde, GSH and carbonyls, and activity of superoxide dismutase and ferric-reducing antioxidant power also were measured.
Results: Serum Hcy was increased significantly after Met treatment and decreased after quercetin supplementation. Meanwhile, serum taurine was increased and serine decreased. However, the content of GSH in serum and liver was decreased in the quercetin-supplemented groups and activities of serum ALT and AST were enhanced in the 1.0% Met and 2.5% quercetin-supplemented groups.
Conclusions: Quercetin is effective in decreasing serum Hcy level in high Met-fed rats and one of possible mechanisms is associated with increased transsulfuration of Hcy. Quercetin can acts as a prooxidant at high intake levels.

Introduction

Homocysteine (Hcy), one of thiol-containing amino acids, is derived from the methionine (Met) metabolism. Two different pathways are involved in its metabolism. The first is remethylation, in which Hcy is recycled to methionine under the catalysis of methionine synthase or betaine-homocysteine methyltransferase (BHMT). The second is transsulfuration, in which Hcy is condensed with serine to form cystathionine under the catalysis of cystathionine-beta-synthase (CBS). The cystathionine is subsequently hydrolyzed to form cysteine (Cys), which is required for glutathione (GSH) synthesis or decomposed finally to form taurine [1–3].

It has been reported that elevated plasma Hcy concentration is an important risk factor for vascular diseases [4–6]. One of the possible mechanisms is associated with increased lipid peroxidation. It is proposed that the thiol group of Hcy undergoes auto-oxidation in vivo and generates reactive oxygen species. Consequently, oxidative stress results and leads to the damage of vascular endothelial cells [7,8]. Some evidences showed that lowering Hcy by nutrition interventions might offer preventive or therapeutic benefits against cardio- and cerebrovascular diseases, although controversy still exists [9–14]. Several dietary factors, including folate, vitamins B_6 and B_{12}, and betaine have been demonstrated to be effective in decreasing plasma Hcy level [10–14]. However, possible side effects of high doses of B vitamins on atherosclerosis have been hypothesized [15]. Therefore, it is necessary to seek new alternatives in treating hyperhomocysteinemia. Recently, it was reported that catechin, one of the main components in teas, also was effective in reducing plasma Hcy levels in mice fed a Met-enriched diet [16]. Quercetin, a common member of the flavonoids family, is well

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known for its antioxidant, antiinflammatory, antidiabetic, and cardioprotective actions and is similar to catechin in chemical structure [17–21]. Previously, a study conducted in our laboratory demonstrated that the *Bhmt* gene, which is known to code for BHMT in Met metabolism, was downregulated, whereas the *Cth* gene encoding for cystathionine gamma-lyase was upregulated in expression by quercetin treatment in rats [22]. Therefore, we hypothesize that quercetin may have potential to combat hyperhomocysteinemia.

The present study investigated the effects of quercetin on Hcy metabolism in rats fed a Met-enriched diet and probed into the mechanisms possibly involved. Additionally, the changes of hepatic antioxidant status after quercetin administration also were measured in order to validate the antioxidant action of quercetin.

Materials and methods

Chemicals

L-Met, Hcy, 4-chloro-7-sulfobenzofurazan ammonium salt (SBD-F), GSH, *N*-acetyl-Cys, L-Cys, 2,4,6-tripyridy-*s*-triazine (TPTZ), β-glucuronidase type H-2 (from Helix pomatia), quercetin, and isorhamnetin were obtained from Sigma-Aldrich, Inc (St Louis, MO, USA). All other chemicals were of the highest grade available.

Animals and experimental protocol

Forty male Wistar rats, weighing 177 g to 198 g, were purchased from the Laboratory Animal Center (Chinese Academy of Military Medical Science, Beijing, P.R. China) and housed individually in stainless-steel cages in a well-ventilated room with a temperature between 18°C and 24°C and relative humidity between 40% and 60%. The light/dark cycles were alternated every 12 h. Food and tap water were provided ad libitum. Dietary intake and body weight were recorded daily. After being acclimatized on a polyphenol-free semisynthetic diet (AIN-93 formula) [23] for 5 d, the rats were divided randomly into five groups, and maintained for 6 wk on the following diets: control diet (AIN-93 diet), 1.0% Met-supplemented AIN-93 diet, 1.0% Met and 0.1% quercetin-supplemented AIN-93 diet, 1.0% Met and 0.5% quercetin-supplemented AIN-93 diet, and 1.0% Met and 2.5% quercetin-supplemented AIN-93 diet. We chose 1.0% Met as the loading dose to induce hyperhomocysteinemia based on studies conducted by Vele-Carrasco et al. and Toue et al. [24,25]. The quercetin was supplemented in the range from 0.1% to 2.5% in order to test whether the action of quercetin was dose-dependent. At the end of the experiment, all rats were fasted overnight and blood samples were collected from the orbital plexus under ether anesthetization. The serum was separated and stored at −20°C. Rat livers also were sampled immediately, cleaned up, and snap-frozen at −80°C before analysis. The experimental protocol was approved by the Department of Scientific Management of the institute and in accordance with the current Chinese legislation on the care and use of laboratory animals.

Serum contents of quercetin and its metabolites

Quercetin and its metabolites in rat serum were determined by a high performance liquid chromatography (HPLC) procedure described by Chen et al. [26]. An aliquot of 150 μL of serum and 100 μL of β-glucuronidase (type H-2) solution in 0.1 M/L acetate buffer containing 37 units of sulfatase and 585 units of β-glucuronidase were incubated with 15 μL of 0.3 g/L ascorbic acid at 37°C for 4 h. Hydrolyzed metabolites were extracted with 265 μL of ethyl acetate, dried under an N_2 flow and reconstituted in 200 μL of methanol. Finally, 40 μL was injected into a symmetry C8 HPLC column (5 um, 3.9 × 150 mm, Waters Corporation, USA). Elution was performed using methanol as solvent A and 0.5% orthophosphoric acid in acetonitrile as solvent B. The gradient for B was as follows: from 100% to 45% in 3 min, from 45% to 35% in 5 min, from 35% to 100% in 5 min, and finally in isocratic condition (100% B) for 10 min. The flow rate was 0.6 mL/min. Elutes were monitored at 370 nm using a 2998 photodiode array detector (Waters Corporation, USA).

Serum contents of Hcy, Cys, Met, taurine, serine, and GSH

Serum Hcy, Cys, and GSH were analyzed according to the method reported by Krijt et al. [27]. Briefly, an aliquot of 100 μL of serum was mixed with 30 μL of sodium borohydride and 5 μL of *N*-octanol. *N*-acetyl-Cys was used as an internal standard. Deproteinization was achieved by the addition of 100 μL of 0.6 M/L perchloric acid containing 1 mM/L ethylenediaminetetraacetic acid (EDTA). Precipitated proteins were removed by centrifugation at 15000 g for 10 min, and 50 μL of supernatant was mixed with 10 μL of 1.55 M/L NaOH and 125 μL of 0.125 M/L borate-EDTA buffer (47.67 g boric acid in 1.0 L of 4 M/L EDTA-2Na), and incubated with a derivatization solution containing 50 μL of 1.0 g/L SBD-F in the borate-EDTA buffer for 1 h at 60°C in the dark. After derivatization, the samples were cooled on ice and protected from light, and 40 μL was injected into a symmetry C_{18} HPLC column (5 um, 3.9 × 150 mm; Waters Corporation, USA). Isocratic elution was carried out with a mobile phase of 0.08 M/L acetic acid containing 2% methanol (pH 4.0) at a flow rate of 0.8 mL/min for 12 min. Elutes were monitored using a 2475 fluorescence detector (Waters Corporation, USA). The fluorescence intensities were measured with excitation at 390 nm and emission at 470 nm. Serum Met, taurine, and serine were measured by an automatic amino acid analyzer (Hitachi L-8800, Tokyo, Japan) based on cation-exchange chromatography and postcolumn reaction with ninhydrin reagent.

Serum activities of alanine transaminase and aspartate transaminase

Serum activities of alanine transaminase (ALT) and aspartate transaminase (AST) were measured using commercial assay kits purchased from Biosino Biotechnology and Science Inc (Beijing, P.R. China).

Hepatic antioxidant capacity, SOD activity, and contents of GSH, malondialdehyde, and carbonyls

The ferric-reducing antioxidant power (FRAP) assay described by Benzie and Strain was followed to analyze hepatic antioxidant capacity [28]. The activity of superoxide dismutase (SOD) was measured using an assay kit purchased from Jiancheng Bioengineering Institute (Nanjing, P.R. China). The contents of GSH and malondialdehyde (MDA) were assayed spectrophotometrically by the reaction with 5, 5'-dithiobis-2-nitrobenzoic acid or thiobarbituric acid, respectively [29]. The content of carbonyls in liver tissue was determined by the method developed by Sundari et al. and Levine et al. [30,31]. Liver samples (200 mg) were first homogenized on ice in phosphate buffer (50 mM/L, pH 7.4) containing digitonin (0.1%, w/v), EDTA (1 mM/L), streptomycin sulfate (1%, w/v), phenylmethyl-sulfonyl fluoride (40 μg/mL), leupeptin (5 μg/mL), pepstatin (7 μg/mL), and aprotinin (5 μg/mL). After centrifugation at 8000 g for 10 min at room temperature, the supernatant was derivatized with dinitrophenyl hydrazine (10 mM/L) in HCl (2.5 M/L) and incubated for 1 h in the dark with intermittent vortexing. After sequential treatment with trichloroacetic acid (20%) and ethanol:acetate (1:1, v:v) mixture, the precipitate was dissolved in 2 mL guanidine hydrochloride (6 M/L) and incubated at 37°C for 10 min. The absorbances were obtained at 370 nm against corresponding HCl-treated samples as blanks.

Statistic analysis

The results are expressed as mean and SD. The differences among groups were examined by one-way ANOVA, followed by least-significant difference test. The level of significance was set at $P < 0.05$.

Results

Dietary intake, body weight gain, and ratio of liver weight to body weight

No significant difference was found in dietary intake among the five groups during experimental period. The body weight gain in the rats fed the 1.0% Met and 2.5% quercetin-supplemented diet decreased significantly compared with those fed the control, 1.0% Met, 1.0% Met and 0.1% quercetin, or 1.0% Met and 0.5% quercetin-supplemented diets ($P < 0.05$; Table 1). The ratio of liver weight to body weight was significantly increased in the rats fed the 1.0% Met and 2.5% quercetin-supplemented diet compared with those fed other diets ($P < 0.05$; Fig. 1).

Serum quercetin and its metabolites

No peaks corresponding to quercetin aglycone or its metabolites were detected on HPLC chromatograms for the serum samples from the control or 1.0% Met-supplemented groups. On the other hand, two distinct peaks corresponding to quercetin