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Anti- and prooxidant effects of chronic quercetin administration in rats

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Abstract

The present study was performed to investigate the effects of chronic administration of quercetin on lipid peroxidation and glutathione concentration in rat liver. Male Sprague–Dawley rats were divided into two groups, one of which was fed a normal diet and the other a vitamin E-free diet. Each of these groups was divided further into three subgroups and treated with quercetin administered orally at either 2 or 20 mg/day or with vehicle for 4 weeks. The concentrations of α-tocopherol in serum and liver increased following quercetin treatment, and these increases were significantly greater in rats maintained on a vitamin E-free diet. Quercetin significantly decreased the concentration of malondialdehyde (an indicator of lipid peroxidation) in the liver and this decrease was more pronounced in vitamin E-deprived rats than in those maintained on a normal diet (55–60% and 25–35% decrease in malondialdehyde concentrations, respectively). Quercetin treatment decreased the glutathione concentration and glutathione reductase activity (40 and 34%, respectively) in the liver significantly and to a similar extent in vitamin E-deprived and -undeprived rats. Collectively, these results suggest that quercetin may act not only as an antioxidant, but also as a prooxidant in rats.

Keywords: Quercetin; Antioxidant; Prooxidant; Glutathione; Malondialdehyde

1. Introduction

Several epidemiological studies have supported the hypothesis that the antioxidant actions of flavonoids may reduce the risk of developing cancer or cardiovascular disease (Sesso et al., 2003). Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a member of the flavone family and is found in many foods, including vegetables, tea, fruit, and wine (Hertog et al., 1993, 1996; Frankel et al., 1993). Flavones have the potential to function as antioxidants, depending on their structure, including the degree of hydroxyl substitution (–OH) and the presence of a catechol-type B ring (Rice-Evans et al., 1996). The antioxidant properties of quercetin might be due to an ability to chelate transition metal ions, such as Fe^{2+} and Cu^{2+}, catalyze electron transport, and scavenge free radicals (Sestili et al., 1998; Chopra et al., 2000; Pedrielli and Skibsted, 2002). The antioxidant actions of quercetin are also associated with the maintenance and regeneration of α-tocopherol, although the precise mechanism underlying this action of quercetin is unclear (Zhu et al., 1999; Van Acker et al., 2000; Hwang et al., 2000).

Recently, a great deal of attention has focused on how the chemical structures of the flavonoids are related to their biological properties, particularly their antioxidant activity (Rice-Evans et al., 1996; Arora et al., 1998; Silva et al., 2002). Most antioxidants including quercetin can interact with other molecules or act as enzyme co-factors. The results of several in vitro studies have suggested that, in addition to acting as an antioxidant, quercetin has prooxidant effects (Laughton et al., 1989; Cao et al., 1997; Galati et al., 1999, 2001). However, to date, few in vivo studies have assessed whether chronic administration of high doses of quercetin produces prooxidant effects. Therefore, the present study was performed to investigate the effects of chronic administration of quercetin on lipid peroxidation and glutathione concentration in rat liver in vivo. The effects of quercetin treatment were compared between rats maintained on a normal and a vitamin E-free diet.

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2. Materials and methods

2.1. Preparation of liver homogenate

Male Sprague–Dawley rats (250–260 g; Daehan Biolink, Chungbuk, Korea) were housed individually in cages (22 ± 2 °C, 40–50% relative humidity) under controlled lighting (12-h light/dark cycle). Rats were fed a standard diet [AIN 93M containing 75 IU of vitamin E (all-rac-α-tocopheryl acetate)/kg diet; Dyets, Bethlehem, PA, USA] and allowed free access to water. After an adaptation period, the rats were divided into two groups, one of which was maintained on a normal diet and another that was fed a vitamin E-free diet (Dyets). Subsequently, each group was subdivided randomly into three treatment groups. Quercetin (Sigma, St. Louis, MO, USA) was dissolved in corn oil and administered orally to two of the three groups at 2 and 20 mg/day for 4 weeks, respectively. Rats in the remaining (control) group were given the vehicle alone. Animal care in this study conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

After 4 weeks, rats were anesthetized by intravenous injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Their livers were isolated, blotted, weighed, frozen in liquid nitrogen and stored at −70 °C until assayed. Tissue was homogenized in 0.1 M phosphate–EDTA buffer (pH 7.4) with 25% HPO_3 at 4 °C.

2.2. Measurement of quercetin and α-tocopherol concentrations

The concentrations of quercetin and α-tocopherol were analyzed by high-performance liquid chromatography (HPLC). To quantify quercetin concentrations, 200-μl aliquots of serum were acidified (pH 4.9) with 0.58 M acetic acid and hydrolyzed with 1000 U β-glucuronidase and 500 U sulfatase for 1 h at 37 °C. Extraction was carried out twice with 8.5 volumes of acetone. The aqueous and organic phases were separated by centrifugation and the upper (organic) phase was evaporated under nitrogen gas. The remaining residue was dissolved in 200 μl of methanol and injected into the HPLC system. The mobile phase was filtered through a 0.45-μm nylon filter and sonicated for 5 min. The mobile phase consisted of 73% water/H_3PO_4 (99.5:0.5, v/v, solvent A) and 27% acetonitrile (solvent B), and the gradient condition was 40% solvent A and 60% solvent B with a flow rate of 1.5 ml/min at an absorbance of 390 nm.

To quantify α-tocopherol concentrations in serum and liver homogenates, sample was deproteinized with ethanol containing 0.01% ascorbic acid. Extraction was carried out twice with 1 ml of *n*-hexane. The separation and injection procedures were the same as described above for quercetin. The mobile phase (methanol/water, 95:5, v/v) was processed at a flow rate of 1.5 ml/min at 290 nm.

The HPLC system consisted of a Waters 501 pump (Waters, Watford, UK) fitted with a UV spectrophotometer (Shimadzu, Kyoto, Japan). Samples were analyzed on a 4.6 × 250 mm RP C-18 Mightysil column (5 μm) (Kanto Chemical, Tokyo, Japan) and HPLC-grade quercetin and α-tocopherol (Sigma) were used as standards.

2.3. Measurement of lipid peroxidation

Lipid peroxidation was determined by measuring the concentration of malondialdehyde in the liver homogenates according to the method of Ohkawa et al. (1979). Liver homogenates were mixed with 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH), and 1.5 ml of a 0.8% aqueous solution of thiobarbituric acid. The volume of the resultant solution was made up to 4.0 ml with distilled water and heated for 60 min at 95 °C. After cooling under tap water, 1.0 ml of distilled water and 5.0 ml of a mixture of *n*-butanol and pyridine (15:1, v/v) were added before shaking the samples vigorously. After centrifugation (3500 × *g*, 10 min), absorbance at 532 nm of the organic layer was measured with a spectrophotometer (model DU 600, Beckman Coulter, Fullerton, CA, USA). Lipid peroxidation was calculated from the standard curve using the malondialdehyde tetrabutylammonium salt and expressed as concentration of nmol malondialdehyde per mg of protein.

2.4. Measurement of concentration of glutathione, and activities of glutathione peroxidase and reductase

The concentration of glutathione was measured with a spectrophotometer (412 nm) using the 5,5′-dithiobis(2-nitrobenzoic acid), DTNB–glutathione disulfide reductase recycling assay for glutathione based on the method of Anderson (1985). Glutathione concentration was expressed as concentration of glutathione per mg protein. Activities of glutathione peroxidase and reductase were determined with a spectrophotometer by measuring the rate of NADPH oxidation at 340 nm, based on the methods of Flohe and Gunzler (1984) and Carlberg and Mannervik (1985). The substrates used in this study were H_2O_2 and oxidized glutathione (GSSG) for glutathione peroxidase and reductase, respectively. Activity was expressed as concentration of nmol $NADP^+$/min/mg protein. Protein concentrations were measured using the method of Bradford (1976).

2.5. Statistical analyses

All values are expressed as means ± S.D. Data were analyzed by unpaired Student's *t*-test or one-way analysis of variance followed by Dunnett's multiple comparison test (Sigma Stat, Jandel, San Rafael, CA, USA). For all comparisons, differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Serum concentrations of quercetin

The concentrations of quercetin in serum from rats treated with 2 or 20 mg/day quercetin for 4 weeks while on a normal diet were 1.83 ± 0.16 and 13.64 ± 0.24 μg/ml, respectively. The concentration of quercetin in the serum of vitamin E-deprived rats was similar to that of undeprived controls (1.24 ± 0.07 and 13.55 ± 0.24 μg/ml in rats treated with 2 or 20 mg/day quercetin, respectively).

3.2. Concentrations of α-tocopherol in serum and liver

In serum, the basal concentration of α-tocopherol in vitamin E-deprived rats (0.60 ± 0.04 ng/ml, $P<0.05$) was significantly lower than that in the undeprived controls (1.82 ± 0.32 ng/ml, Fig. 1A). In vitamin E-deprived rats, quercetin (2 or 20 mg/day) significantly increased the concentration of α-tocopherol in a dose-dependent manner as compared to vehicle-treated animals, while quercetin concentrations did not increase significantly in undeprived rats.

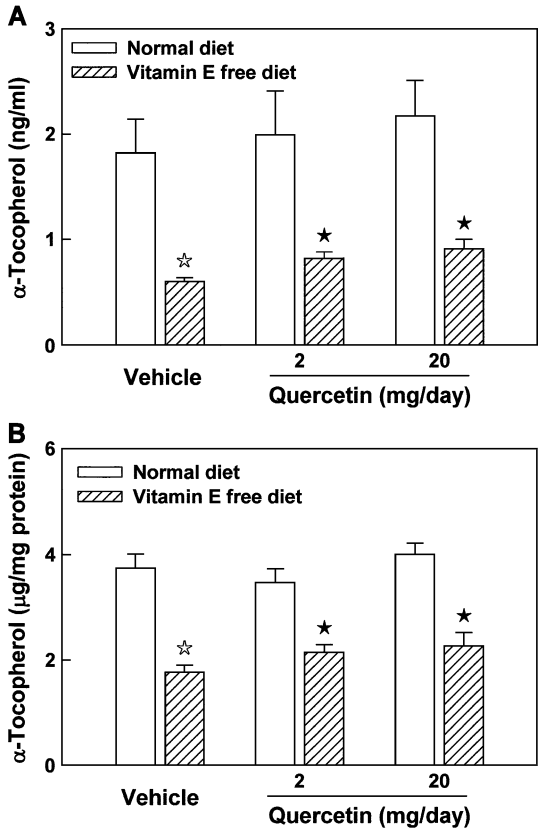

Fig. 1. Concentration of α-tocopherol in serum (A) and liver (B). Each group was subdivided into three groups that were treated for 4 weeks with either 2 or 20 mg/day or with vehicle. Values are means ± S.D. ($n=6–9$). ☆$P<0.05$, significantly different from undeprived rats treated with vehicle. ★$P<0.05$, significantly different from vitamin E-deprived rats treated with vehicle.

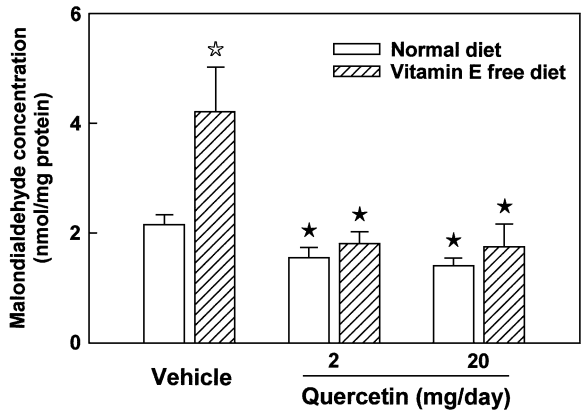

Fig. 2. Concentration of malondialdehyde in liver. Each group was subdivided into three groups that were treated for 4 weeks with either 2 or 20 mg/day or with vehicle. Values are means ± S.D. ($n=6–9$). ☆$P<0.05$, significantly different from undeprived rats treated with vehicle. ★$P<0.05$, significantly different from the respective vehicle-treated group.

In the liver, the concentration of α-tocopherol in vitamin E-deprived rats (1.77 ± 0.14 ng/ml, $P<0.05$) was significantly lower than that in the undeprived controls (3.74 ± 0.27 ng/ml, Fig. 1B). The effects of quercetin treatment on α-tocopherol in the liver were similar to those in serum.

3.3. Lipid peroxidation

The basal concentration of malondialdehyde in the liver of vitamin E-deprived rats was significantly ($P<0.05$) greater than that in undeprived rats (4.22 ± 0.82 and 2.16 ± 0.18 nmol/mg protein, respectively, Fig. 2). In undeprived rats, the concentration of malondialdehyde was significantly lower following quercetin treatment (27.8% and 34.3% for 2 and 20 mg/day, respectively, compared with the respective vehicle-treated groups, $P<0.05$). This effect was more pronounced in vitamin E-deprived rats (56.9% and 58.1% for 2 and 20 mg/day, respectively, $P<0.05$).

3.4. Glutathione concentration

The basal concentrations of glutathione in the livers of vitamin E-deprived and -undeprived rats were 15.2 ± 1.21 and 16.93 ± 1.56 nmol/mg protein, respectively (Fig. 3A). Quercetin treatment (2 or 20 mg/day) significantly decreased the concentration of glutathione both in vitamin E-deprived and -undeprived rats ($P<0.05$).

3.5. Activities of glutathione peroxidase and reductase

The basal activity of glutathione peroxidase in the liver was similar in vitamin E-deprived and -undeprived rats (110.55 ± 28.75 and 106.66 ± 13.88 nmol $NADP^+$/min/mg protein, respectively, Fig. 3B). Quercetin treatment caused a slight increase (not significant) in the activity of glutathione peroxidase in the same rats.

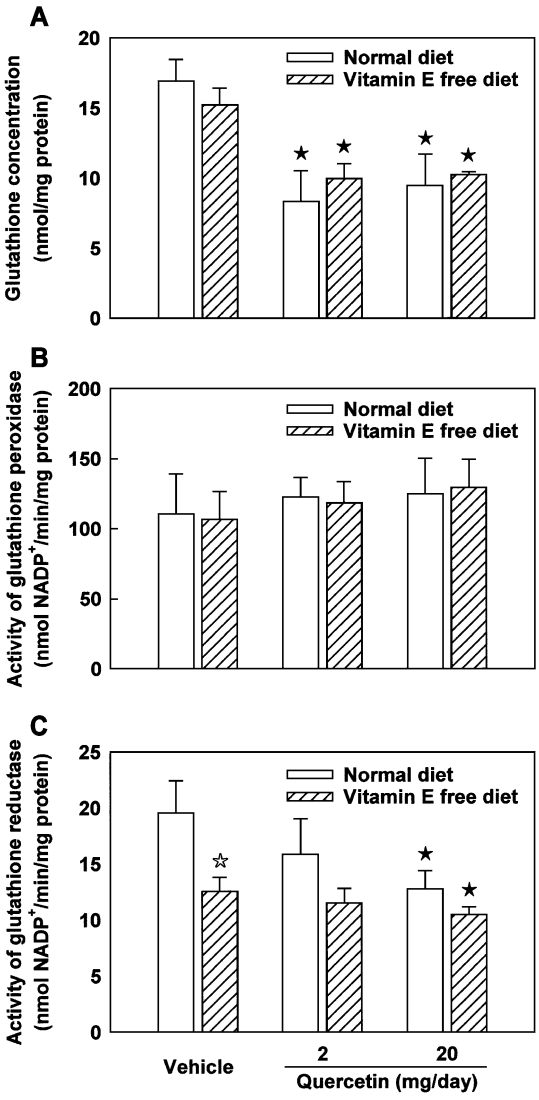

Fig. 3. Concentration of glutathione (A), and activities of glutathione peroxidase (B) and reductase (C) in the liver. Each group was subdivided into three groups that were treated for 4 weeks with either 2 or 20 mg/day or with vehicle. Values are means ± S.D. ($n=6-9$). $^{\star}P<0.05$, significantly different from undeprived rats treated with vehicle. $^{\star}P<0.05$, different from the respective vehicle-treated group.

The basal activity of glutathione reductase in vitamin E-deprived rats was significantly lower than that in undeprived rats (12.57 ± 1.25 and 19.54 ± 2.89 nmol $NADP^+$/min/mg protein, Fig. 3C). Quercetin treatment decreased glutathione reductase activity in a dose-dependent manner both in vitamin E-deprived and -undeprived rats ($P<0.05$ for 20 mg/day).

4. Discussion

The present study was carried out to investigate the anti- and prooxidant effects of quercetin in the rat liver. Quercetin is found in a variety of foods, such as vegetables, tea, fruit and wine (Frankel et al., 1993; Hertog et al., 1993, 1996). Quercetin can be accumulated and maintained in the body more easily than other flavonoids, even though it is hydrophilic (Manach et al., 1997). The metabolites of quercetin also can be accumulated in the body even after short-term ingestion of quercetin-rich vegetables (Manach et al., 1997; Moon et al., 2000).

The basal concentrations of α-tocopherol in the serum and liver from vitamin E-deprived rats were significantly lower than those in undeprived animals. Interestingly, the concentrations of α-tocopherol in the serum and liver increased following quercetin treatment, and this effect was more pronounced in vitamin E-deprived rats. The mechanism by which quercetin increases the concentration of α-tocopherol is not clear, although one possibility is that α-tocopherol could be regenerated by quercetin from α-tocopheryl radicals in the body (Van Acker et al., 2000; Pedrielli and Skibsted, 2002). In the present study, quercetin significantly decreased the concentration of malondialdehyde, an indicator of lipid peroxidation, in the liver homogenates. Among the numerous pathological events that are caused by an imbalance between oxidative damage and antioxidant defense systems, lipid peroxidation is a well-known example of oxidative damage that affects cell membranes, lipoproteins, and other lipid-containing structures under conditions of oxidative stress. Therefore, the fact that quercetin decreased lipid peroxidation might mean that quercetin has an antioxidant effect. The inhibitory effect of lipid peroxidation by quercetin was more pronounced in vitamin E-deprived rats than in undeprived animals. We did not investigate why quercetin was more effective in vitamin E-deprived rats than in undeprived rats, but it is possible that lipid peroxidation may be more sensitive to inhibition by quercetin when the level of vitamin E in the body is restricted. Alternatively, α-tocopherol may be regenerated easily by quercetin from α-tocopheryl radicals under conditions of vitamin E deficiency. These results suggest that chronic administration of quercetin can produce antioxidant effects in rats and that these effects are exaggerated in rats that are deprived of vitamin E.

In the present study, chronic administration of quercetin significantly decreased glutathione concentration and glutathione reductase activity both in vitamine E-deprived and -undeprived rats. These observations are in line with the results of a previous in vitro study, which found that flavonoids such as quercetin and myricetin caused concentration-dependent decreases in nuclear glutathione concentration in isolated rat liver nuclei (Sahu and Gray, 1996). It has been well known that dysfunction of glutathione metabolism (glutathione concentration or the enzymes involved in glutathione pathway) causes oxidative damage (Comporti et al., 1991). A dysfunction of glutathione metabolism has been also noted in most neurodegenerative diseases such as Huntington's disease (Cruz-Aguado et al., 2000), Alzheimer's disease (Adams et al., 1991) and Parkinson's disease (Bharath et al., 2002). Hence, the upregulation of glutathione system has been of importance in antioxidant defense

system and good health status. How quercetin decreases the glutathione concentration and glutathione reductase activity is still not clear, although a possibility has been suggested. That is, the C4-keto moiety and the C2=C3 double bond in quercetin facilitate formation of its *o*-quinoid type metabolites, quinones and quinone methides (Awad et al., 2001). These metabolites could be removed by the glutathione that serves as an antioxidant defense system in the body (Monks and Lau, 1998; Awad et al., 2000; Boersma et al., 2000) and would result in a decrease in glutathione concentration. Accordingly, the decrease of glutathione concentration and glutathione reductase activity by quercetin imply the weakness of antioxidant defense system in the body, and these suggest that quercetin may cause prooxidant action.

Collectively, the results of the present study indicate that chronic administration of quercetin in rats caused the inhibition of lipid peroxidation and the decrease in glutathione concentration and glutathione reductase activity. These results suggest that, in addition to acting as an antioxidant, quercetin may have prooxidant effects.

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