Differential Effects of Flavonoid Quercetin on Oxidative Damages Induced by Hydrophilic and Lipophilic Radical Generators in Hepatic Lysosomal Fractions of Mice

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We evaluated the efficacy of flavonoid quercetin as an antioxidant in hepatic lysosomal fractions of mice using the hydrophilic radical generator 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) and the lipophilic radical generator 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN). Quercetin inhibited lipid peroxidation in lysosomal fractions, measured as thiobarbituric acid reactive substances (TBARS), and inhibited the release of lysosomal enzymes more obviously against AAPH than against AMVN. Whereas inhibitory effects of quercetin on lipid peroxidation were weaker than those of the synthetic lipophilic antioxidant 2,6-di-*tert*-butyl-*p*-cresol (BHT), lysosomal fractions preloaded with quercetin effectively quenched 1,1-diphenyl-2-picrylhydrazyl radicals, similar to BHT. Rutin, a glycoside of quercetin, was less potent than quercetin in these experiments. These findings suggest that quercetin could have potent antioxidative activity in the spaces between an aqueous phase and a lipid phase in biological systems owing to the localization within membranes as well as evident antioxidative activity.

Key words —— quercetin, lipid peroxidation, antioxidant, flavonoid, radical scavenger, biomembrane

INTRODUCTION

Polyphenols such as flavonoids in the diet have possible beneficial effects on human health.[1] Quercetin and its derivatives form one of the major flavonoid groups distributed in plant foods like onions and teas. Antioxidant activities of flavonoids may be involved in the correlation between a high intake of polyphenols and a low risk of disorders such as cardiovascular disease.[2]

We have previously suggested that the consumption of quercetin may stabilize biomembranes in mice by reducing lipid peroxidation and scavenging free radicals.[3] In addition, subcellular distribution of quercetin, which is liable to be influenced by drug metabolism, could be an important factor in modifying the efficiency of antioxidant activities of ingested substances in biological systems. In this study we aimed to elucidate the differences in antioxidant activities of quercetin between aqueous and lipid environments using the hydrophilic radical generator 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) and the lipophilic radical generator 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) *in vitro*.

MATERIALS AND METHODS

AAPH and AMVN were purchased from Wako (Osaka, Japan), and other chemicals were obtained from Nakalai Tesque (Kyoto, Japan). The ddY strain male mice (body weight: 30–35 g) were obtained from the Shizuoka Laboratory Animal Center (Hamamatsu, Japan), and were kept in wire cages under temperature-controlled conditions before use.

Hepatic lysosomal fractions were prepared according to the method described previously.[3]

The effects of phenolic compounds such as quercetin, rutin, and 2,6-di-*tert*-butyl-*p*-cresol (BHT) on radical generator-induced lipid peroxidation in lysosomal fractions were tested by measuring thiobarbituric acid reactive substances (TBARS) by spectrophotometry[3] in the presence of 50 mM AAPH or 1 mM AMVN.

Assays for the release of acid phosphatase and *β-N*-acetylglucosaminidase from lysosomes were carried out by incubating lysosomal suspensions with phenolic compounds in the presence of 50 mM AAPH or 1 mM AMVN according to the method previously reported.[3] The effects of the test compounds on lysis were calculated as a percentage of control.

Quenching activity of phenolic compounds against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was measured by spectrophotometry. One ml sample solutions, phenolic compounds in dimethyl sulfoxide or lysosomal suspensions preloaded with

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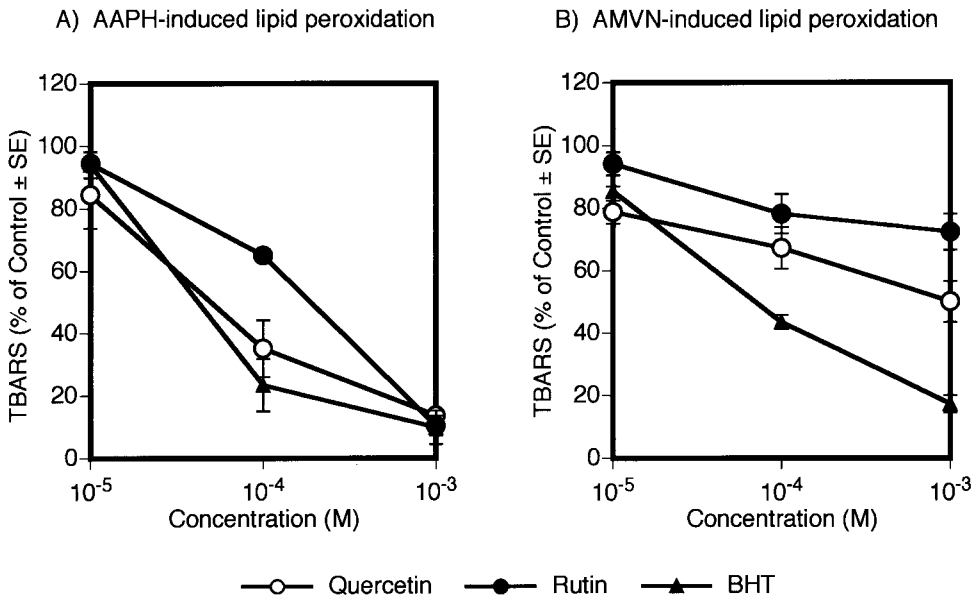

Fig. 1. Effects of Polyphenols on Lipid Peroxidation Induced by Free Radical-Generators in Lysosomal Fractions
A: in the presence of 50 mM AAPH; B: in the presence of 1 mM AMVN.

phenolic compounds, were incubated with 2 ml ethanol solution of 0.25 mM DPPH radicals and 2 ml 0.1 M acetate buffer (pH 5.5) for 45 min at 37°C, and then absorbance was measured at 517 nm. For this experiment, lysosomal suspensions were preincubated with 1 mM phenolic compounds for 30 min, and centrifuged at 12000 × *g* for 10 min. Then the pellets were washed in 0.15 M KCl-5 mM Tris buffer (pH 7.4), centrifuged, and re-suspended in 0.1 M acetate buffer (pH 5.5).

All values are expressed as the mean ± S.E. Significant differences were evaluated by Student's paired *t*-test with $p < 0.05$.

RESULTS AND DISCUSSION

AAPH and AMVN are azo-compounds that generate peroxide radicals after thermal homolysis in aqueous phase and lipid phase, respectively.[4] We evaluated the antioxidative activity of quercetin by measuring lipid peroxidation as TBARS in lysosomal fractions in the presence of these free radical generators. Figure 1 shows that the phenolic compounds tested in this experiment inhibited dose-dependently lipid peroxidation induced by radical generators. The degree of the inhibition by phenolic compounds appears to be strongest in the order of BHT > quercetin > rutin. The differences were evident when AMVN was used, suggesting that the affinities of phenolic compounds and radical generators for the lipid phase influenced their antioxidative potency.

BHT is a synthetic lipophilic antioxidant used widely in foods and in laboratories. Quercetin is more lipophilic than its glycoside rutin, and has been reported to localize near the surface of lipid bilayers of liposomal membranes.[5] On the other hand we previously demonstrated that these flavonoids scavenged peroxyl radicals more efficiently than BHT by measuring AAPH-induced chemiluminescence.[3] Therefore, the solubility of flavonoids in the lipid phase could be highly relevant to the inhibition of lipid peroxidation occurring within biomembranes.

The significance of the lipid solubility of antioxidants was also seen when DPPH radical-scavenging abilities were compared. The activity of BHT was weaker than those of quercetin and rutin in aqueous solution (Fig. 2A), which supports the previous results in our chemiluminescence assay. However, lysosomal fractions preincubated with BHT showed equally quenched DPPH radicals to those pretreated with quercetin, whereas the fractions pretreated with rutin showed a moderate radical-scavenging activity (Fig. 2B).

Lysosomes contain many hydrolases within their membranes. Quercetin, tested *in vitro*[6] and *ex vivo*,[3] inhibited the release of hydrolases from lysosomal vesicles exposed to oxygen free radicals probably owing to the inhibition of oxidative damage of lysosomal membranes. In this study (Table 1) both quercetin and rutin provided more effective protection

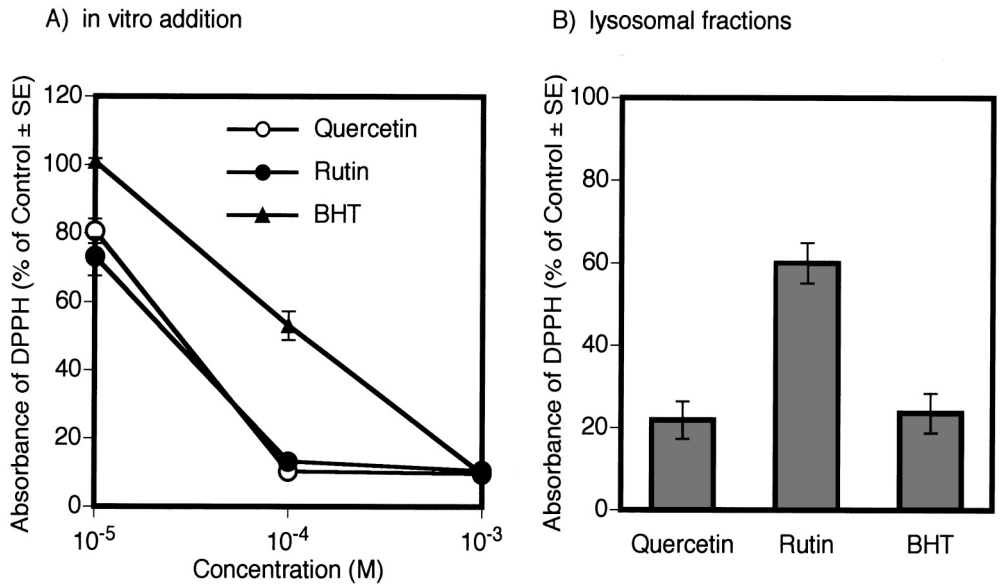

Fig. 2. DPPH Radical-Scavenging Activity of Polyphenols
The absorbance of DPPH radicals was measured after mixing with polyphenols (A) or with lysosomal fractions preincubated with polyphenols (B).

Table 1. Effects of Phenolic Compounds on the Release of Lysosomal Enzymes in the Presence of Hydrophilic or Lipophilic Radical Generators

A: AAPH-induced release

Compounds		Enzyme activity (% of control ± S.E.)	
		Acid phosphatase	*β-N*-Acetylglucosaminidase
Quercetin	10^{-5} M	57.0 ± 5.2*	57.9 ± 0.8*
	10^{-4} M	38.1 ± 2.6*	30.1 ± 6.2*
Rutin	10^{-5} M	62.7 ± 5.2*	61.5 ± 5.4*
	10^{-4} M	37.7 ± 3.3*	29.2 ± 7.7*
BHT	10^{-5} M	95.5 ± 2.2	68.0 ± 2.7*
	10^{-4} M	49.9 ± 2.5*	58.9 ± 7.6*

B: AMVN-induced release

Compounds		Enzyme activity released (% of control ± S.E.)	
		Acid phosphatase	*β-N*-Acetylglucosaminidase
Quercetin	10^{-4} M	113.7 ±17.2	43.1 ± 5.3*
Rutin	10^{-4} M	111.0 ± 8.8	101.6 ± 2.1
BHT	10^{-4} M	96.6 ± 3.8	87.6 ± 5.4

Control was determined in the presence of DMSO instead of the solution of phenolic compounds. $^{*}p < 0.05$ ($n = 3$) differs significantly from the control by Student's paired *t*-test.

against hydrophilic generator AAPH-induced lysosomal lysis than against lipophilic generator AMVN-induced lysis. The flavonoids protected lysosomal membranes more effectively against free radicals generated in the aqueous phase. BHT, however, did not antagonize the enzyme release induced by lipophilic radical generator as effectively as quercetin, irrespective of its potent antioxidant activity, suggesting that factors other than lipid peroxidation may be involved in the suppressive effect of flavonoids. Decharneux *et al.*[6] proposed a more direct action of flavonoids on lysosomal membranes. Additionally, the localization of flavonoids within the membranes may modify membrane fluidity and lipid peroxidation as documented by some investigators.[7,8]

In conclusion, flavonoids may be effective as antioxidants in spaces between the aqueous phase and the lipid phase owing to their localization near the surface in lipid bilayers. Thus, not only evident antioxidative activity, but also the subcellular localization of flavonoids may contribute to their potencies as antioxidants in biological systems.

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