Individual and Combined Antioxidant Effects of Seven Phenolic Agents in Human Erythrocyte Membrane Ghosts and Phosphatidylcholine Liposome Systems: Importance of the Partition Coefficient

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Antioxidant activities of seven phenolic agents against Fe^{2+}-induced lipid oxidation were compared with α-tocopherol, β-carotene, and vitamin C in human erythrocyte membrane ghosts and liposome systems. The antioxidant activity of five test flavonoids followed the order catechin > epicatechin > rutin > quercetin > myricetin in both systems ($p < 0.05$), which was negatively correlated with their partition coefficients. The antioxidant interaction of these phenolic agents with α-tocopherol, β-carotene, or vitamin C in inhibiting Fe^{2+}-induced lipid oxidation was examined. Synergistic effects were present in the combinations of α-tocopherol plus caffeic acid, catechin, or epicatechin as well as in all combinations of vitamin C plus phenolic antioxidants. On the basis of the stronger individual and combined effects present in caffeic acid, catechin, and epicatechin, the application of these three phenolic agents with or without α-tocopherol, β-carotene, and vitamin C may provide stronger protective benefits against lipid oxidation, which may be helpful for oxidation-related diseases prevention.

Keywords: *Phenolic agents; synergistic effect; partition coefficient; human erythrocyte membrane ghosts*

INTRODUCTION

The oxidation induced by free radicals can result in cell membrane disintegration, membrane protein damage, and DNA mutation (Slater, 1984; Halliwell, 1994), which can further initiate or propagate the development of many diseases such as cancers, liver injury, and cardiovascular disease (Yagi, 1987). To prevent or cure these oxidation-related diseases, blocking the generation of free radicals is important. Thus, intake of α-tocopherol, β-carotene, vitamin C, and foods rich in these antioxidant nutrients is encouraged (Haglund et al., 1991; Buring and Hennekens, 1995; Harats et al., 1998). Recently, there has been an increased interest regarding the application of phenolic antioxidants other than α-tocopherol in clinical or preventive nutrition (Hertog et al., 1993; Manach et al., 1995).

Many epidemiological studies have shown that increasing the consumption of foods rich in phenolic antioxidants could reduce the risk of cancer and coronary heart disease (Hertog et al., 1993; Criqui and Ringel, 1994). Like α-tocopherol, the primary protective mechanism of a phenolic antioxidant is to trap and stabilize free radical species (Masuda et al., 1999). Moreover, Saija et al. (1995) and Saskia et al. (1996) indicated that the interaction of flavonoids with biomembranes was an important factor in determining their structure–activity relationship. The interaction of an agent with biomembranes, or the uptake of an agent into the membranes, is strongly related to its lipophilicity, which is expressed as the partition coefficient. Since this coefficient can be measured for every agent, the influence of lipophilicity upon antioxidant activity of flavonoids and other phenolic agents should be determined.

The antioxidant relationship between α-tocopherol and a few phenolic antioxidants has been studied (Terao et al., 1994; Nardini et al., 1997). These authors reported that caffeic acid and epicatechin had a sparing effect on α-tocopherol. It remains unknown whether other phenolic antioxidants also have this sparing effect on α-tocopherol. β-Carotene is also a lipophilic antioxidant, while less attention has been paid to the antioxidant interaction of β-carotene with phenolic antioxidants. Vitamin C is able to regenerate α-tocopherol and showed a synergistic relationship with α-tocopherol (Yin et al., 1993; Harats et al., 1998). Since α-tocopherol is a phenolic antioxidant, it is reasonable to examine the antioxidant interaction of vitamin C with other phenolic antioxidants.

The first purpose of this study was to compare the antioxidant activity of seven phenolic agents (two nonflavonoid phenolic acids, caffeic acid and gallic acid, and five flavonoids, catechin, epicatechin, quercetin, rutin, and myricetin) with each other and with α-tocopherol, β-carotene, and vitamin C in human erythrocyte membrane ghosts and liposome systems. These seven phenolics were selected because they are found in tea, vegetables, and fruits commonly consumed in Taiwan. Second, the antioxidant interaction of the seven phenolic agents with α-tocopherol, β-carotene, or vitamin C in these two systems was studied. The influence of lipophilicity of phenolic agents upon its antioxidant activity was evaluated.

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MATERIALS AND METHODS

Chemicals. The seven phenolic agents used in this study were caffeic acid, catechin, myricetin, epicatechin, gallic acid, quercetin, and rutin. These agents were purchased from Sigma Chemical Co. (St. Louis, MO), and the purity was greater than 99.5%. α-Tocopherol, β-carotene, vitamin C, trichloroacetic acid (TCA), thiobarbituric acid (TBA) ,and chemicals used for liposome and erythrocyte membrane ghosts preparation were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

Liposome Preparation. Phosphatidylcholine (PC) with oleic acid (C18:1, n-9, at Sn-1 position) and linoleic acid (C18: 2, n-6, at Sn-2 position) was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Liposomes (multilamellar vesicles) were prepared from PC (30 mg), cholesterol (12 mg), and dicetyl phosphate (3 mg) at 4 °C as described by Yin et al. (1993). The solvents (chloroform, methanol) used for liposomes preparation were removed and a lipid film was formed by rotary evaporation under N_2 flush. The buffer for liposomes suspension was sodium phosphate buffer (0.05 M, pH 7.2). After preparation, all samples were incubated at 37 °C for oxidation measurement.

Human Erythrocyte Membrane Ghosts Preparation. Human blood was drawn from healthy graduate students in Chungshan Medical & Dental College (Taichung, Taiwan, R.O.C.). The method described by Virgili et al. (1996) was used to prepare erythrocyte membrane ghosts: 50 mL blood was diluted with 150 mL of isotonic buffer solution (10 mM phosphate buffer saline, PBS, pH 7.2). Erythrocytes were separated by centrifugation at 1500*g*, 10 min, washed three times with 10 mL of PBS, and then suspended at 50% hematocrit in PBS containing 10 μL/mL pen−strep (300 mg of penicillin G and 500 mg of streptomycin in 10 mL of distilled water). Membrane ghosts were prepared by suspending these erythrocytes in hypotonic phosphate buffer (5 mM, pH 8.2) for lysis. The hemoglobin-free membranes (ghosts) were pelleted by centrifugation at 20000*g* for 40 min and further washed twice in the same buffer in the presence of phenylmethanesulfonyl fluoride as inhibitor of proteases. The protein concentration in membrane ghosts was determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard. The ghosts were diluted in PBS plus phenylmethanesulfonyl fluoride and pen−strep solution to a protein concentration of 2 mg/mL for different treatments.

α-Tocopherol, β-Carotene, Vitamin C, and Phenolic Agents Analysis in Erythrocyte Membrane Ghosts and Liposomes. The concentrations of α-tocopherol and β-carotene in erythrocyte membrane and prepared liposomes were analyzed by the HPLC method of Palozza and Krinsky (1992). Vitamin C content was analyzed by the method of Dhariwal et al. (1991). The concentrations of seven phenolic agents were analyzed by the method of Lamuela-Raventos and Waterhouse (1993), Terao et al. (1994), and Manach et al. (1995). The concentrations of α-tocopherol, quercetin, and rutin in RBC ghosts were 0.55 ± 0.12, 0.13 ± 0.08, and 0.18 ± 0.10 μM, which were equal to 0.28 ± 0.06, 0.07 ± 0.04, and 0.09 ± 0.05 μmol/mg membrane protein. The concentrations of β-carotene, vitamin C, and other phenolic agents in RBC ghosts were too low to be detected. In liposomes, only 0.08 ± 0.03 μM α-tocopherol was found.

Antioxidant Treatments. For individual effectiveness of antioxidant action, the concentration of α-tocopherol, β-carotene, seven phenolic agents, or vitamin C was 10 μM. For antioxidant interaction experiments, 5 μM α-tocopherol, β-carotene, or vitamin C was combined with 5 μM phenolic agent. On the basis of the lipid solubility, α-tocopherol, β-carotene, or phenolic agent was added into multilamellar vesicles with PC during liposomes preparation. Also, these lipid-soluble antioxidants (10 mg/mL) were suspended in methanol/chloroform (9:1) first and then added into erythrocyte membrane ghosts for the final concentration preparation. The influence of methanol/chloroform residue upon lipid oxidation in erythrocyte membrane ghosts was examined and it was not significant (data not shown). Vitamin C was directly added into sodium phosphate buffer for liposomes or membrane ghosts preparation. The recovery of these antioxidants after they were incorporated into RBC ghosts or liposomes was analyzed. The recovery rate was ≥98.5% for all agents.

Lipid Oxidation Measurement. At the start, 5 μM $FeSO_4$ was added into liposomes or erythrocyte membrane ghosts to induce lipid oxidation. Lipid oxidation was measured by the thiobarbituric acid (TBA) assay as described by Yin et al. (1993) at 0, 36, and 72 h; 1 mL of sample (liposomes or membrane ghosts) was mixed with 0.5 mL of 30% TCA and the mixture was centrifuged at 1400*g* for 5 min at 4 °C; 1 mL of supernatant was mixed with 1 mL of 0.02 M TBA and the mixture was stored in the dark for 20 h at 25 °C. At the end of 20 h, absorbance of the final solution was measured by UV−vis spectrophotometry at 532 nm and recorded as TBA number. The blank for β-carotene-containing samples was a liposome or erythrocyte membrane solution with equal concentration of β-carotene as sample. The interference of pigment from phenolic antioxidants (such as yellow color from myricetin) was very mild and did not affect lipid oxidation measurement. The lipid stability of purchased PC or erythrocyte membrane was examined, and the PC or erythrocyte membrane with TBA number ≤0.01 was used for both liposomes and erythrocyte membrane preparation.

Partition Coefficient Measurement. The method similar to that of Foti et al. (1996) was used to measure the partition coefficient of α-tocopherol, β-carotene, vitamin C, and seven phenolic agents. A solution of 1 mM agent in *n*-octanol/water (1:1) was mixed thoroughly by vortexing 10 min at the highest speed and maintained at 37 °C for 2 h. A UV spectrum was run and the value of the absorbance at the maximum was measured. UV spectra of the *n*-octanol layer were run every 30 min until the absorbance value was constant. A solution of *n*-octanol saturated with water was used as blank. The concentration of agent in *n*-octanol layer was converted from the absorbance. The partition coefficient of an agent was (its concentration in *n*-octanol) ÷ (its concentration in water). The agent with partition coefficient >1 was more lipophilic because its concentration in the *n*-octanol layer was higher.

Statistical Analysis. The effect of each experimental condition was analyzed on liposomes or erythrocyte membrane ghosts from five different preparations ($n = 5$). Data were treated by analysis of variance (ANOVA) and computed using the SAS General Model procedure (SAS Institute, Inc., 1985). Difference among means was determined by the least significance difference test with significance defined at $p \leq 0.05$.

RESULTS

The partition coefficient and individual antioxidant activity of α-tocopherol, β-carotene, vitamin C, and seven phenolic agents are presented in Table 1. The partition coefficients of five test flavonoids followed the order myricetin > quercetin > rutin > epicatechin > catechin ($p < 0.05$). The partition coefficients of caffeic acid and gallic acid were not significantly different ($p > 0.05$). At the concentration of 10 μM, α-tocopherol, β-carotene, vitamin C, and seven phenolic agents significantly delayed Fe^{2+}-induced lipid oxidation when compared with controls in used two systems ($p < 0.05$). The antioxidant activity of five flavonoids followed the order catechin > epicatechin > rutin > quercetin > myricetin in both systems ($p < 0.05$), which was exactly negatively correlated with their partition coefficients. The antioxidant activity of caffeic acid was greater than that of gallic acid in both systems ($p < 0.05$).

The interactions of seven phenolic antioxidants with α-tocopherol, β-carotene, and vitamin C in human erythrocyte membrane ghosts are shown in Tables 2−4. Similar results were also observed in the liposomal model (data not shown). In Table 2, all combinations of 5 μM α-tocopherol plus 5 μM phenolic agent showed

Table 1. Partition Coefficient and Individual Antioxidant Effect of α-Tocopherol, β-Carotene, Vitamin C, and Seven Phenolic Agents upon Fe^{2+}-Induced Lipid Oxidation in Human Erythrocyte Membrane Ghosts (Human RBC Ghosts)[a] and Liposome Systems[a] after a 72-h Incubation at 37 °C

treatment	partition coeff[b]	human RBC ghosts	liposomes
control	*c*	0.535 ± 0.045[i]	0.529 ± 0.033[h]
α-tocopherol	549 ± 9.62[f]	0.126 ± 0.012[a]	0.121 ± 0.010[a]
β-carotene	753 ± 15.45[g]	0.443 ± 0.034[h]	0.451 ± 0.024[g]
vitamin C	*d*	0.367 ± 0.025[g]	0.344 ± 0.021[f]
catechin	0.60 ± 0.072[a]	0.145 ± 0.011[b]	0.145 ± 0.012[b]
epicatechin	0.94 ± 0.174[b]	0.168 ± 0.020[c]	0.172 ± 0.015[c]
myricetin	5.27 ± 0.391[e]	0.342 ± 0.028[g]	0.335 ± 0.028[f]
quercetin	3.84 ± 0.343[d]	0.310 ± 0.029[f]	0.285 ± 0.025[e]
rutin	1.27 ± 0.516[c]	0.224 ± 0.016[d]	0.209 ± 0.023[d]
caffeic acid	0.56 ± 0.052[a]	0.203 ± 0.013[d]	0.192 ± 0.015[d]
gallic acid	0.45 ± 0.057[a]	0.271 ± 0.023[e]	0.274 ± 0.021[e]

[a] TBA number expressed as least-squares mean ± SD, $n = 5$. [b] Data expressed as mean ± SD, $n = 5$. [c] Controls contained no antioxidant agent. [d] The concentration of vitamin C in *n*-octanol was too low to be detected. [a−i] Least-squares means with a common superscript within a column are not different at the 5% level.

Table 2. Combined Effect of α-Tocopherol plus Phenolic Agent upon Fe^{2+}-Induced Lipid Oxidation in Human Erythrocyte Membrane Ghosts Systems after a 72-h Incubation at 37 °C

treatment	concn (μM)	TBA number[a]
controls	*b*	0.519 ± 0.037
α-tocopherol	5	0.193 ± 0.011
	10	0.122 ± 0.010
caffeic acid	5	0.376 ± 0.023
	10	0.208 ± 0.016
α-tocopherol + caffeic acid	5 + 5	0.095 ± 0.010*
catechin	5	0.258 ± 0.017
	10	0.137 ± 0.013
α-tocopherol + catechin	5 + 5	0.081 ± 0.022*
myricetin	5	0.468 ± 0.034
	10	0.331 ± 0.024
α-tocopherol + myricetin	5 + 5	0.252 ± 0.026
epicatechin	5	0.326 ± 0.022
	10	0.164 ± 0.013
α-tocopherol + epicatechin	5 + 5	0.084 ± 0.008*
gallic acid	5	0.381 ± 0.028
	10	0.281 ± 0.018
α-tocopherol + gallic acid	5 + 5	0.118 ± 0.012#
quercetin	5	0.411 ± 0.036
	10	0.291 ± 0.020
α-tocopherol + quercetin	5 + 5	0.209 ± 0.010
rutin	5	0.348 ± 0.021
	10	0.213 ± 0.017
α-tocopherol + rutin	5 + 5	0.183 ± 0.015

[a] TBA number expressed as least-squares mean ± SD, $n = 5$. [b] Controls contained no antioxidant agent. *The TBA number of this combination is significantly lower than that of 10 μM corresponding phenolic agent and also lower than that of 10 μM α-tocopherol at $p < 0.05$. # The TBA number of this combination is significantly lower than that of 10 μM corresponding phenolic agent at $p < 0.05$ but not significantly different from that of 10 μM α-tocopherol ($p > 0.05$).

greater antioxidant effect against lipid oxidation than corresponding phenolic agent at 10 μM treatment alone because of the significantly lower TBA number ($p < 0.05$). Furthermore, the TBA number of 5 μM α-tocopherol plus 5 μM caffeic acid, catechin, or epicatechin was even significantly lower than that of 10 μM α-tocopherol treatment alone ($p < 0.05$). In Table 3, all combinations of 5 μM β-carotene plus 5 μM phenolic agent show a greater antioxidant effect than 10 μM β-carotene treatment alone ($p < 0.05$); however, every combination showed a less protective effect than the corresponding phenolic agent at 10 μM treatment alone ($p < 0.05$). In Table 4, all combinations of 5 μM vitamin C plus 5 μM phenolic agent result in a greater antioxidant effect than 10 μM vitamin C treatment alone ($p < 0.05$) and also greater than the corresponding phenolic antioxidant treatment (10 μM) alone ($p < 0.05$).

Table 3. Combined Effect of β-Carotene Plus Phenolic Agent upon Fe^{2+}-Induced Lipid Oxidation in Human Erythrocyte Membrane Ghosts System after a 72-h Incubation at 37 °C

treatment	concn (μM)	TBA number[a]
controls	*b*	0.520 ± 0.034
β-carotene	5	0.486 ± 0.035
	10	0.432 ± 0.021
caffeic acid	5	0.395 ± 0.014
	10	0.200 ± 0.013
β-carotene + caffeic acid	5 + 5	0.245 ± 0.022†
catechin	5	0.272 ± 0.017
	10	0.131 ± 0.011
β-carotene + catechin	5 + 5	0.169 ± 0.010†
myricetin	5	0.478 ± 0.034
	10	0.345 ± 0.025
β-carotene + myricetin	5 + 5	0.356 ± 0.031†
epicatechin	5	0.302 ± 0.018
	10	0.155 ± 0.012
β-carotene + epicatechin	5 + 5	0.193 ± 0.016†
gallic acid	5	0.374 ± 0.026
	10	0.273 ± 0.021
β-carotene + gallic acid	5 + 5	0.324 ± 0.022†
quercetin	5	0.398 ± 0.025
	10	0.279 ± 0.023
β-carotene + quercetin	5 + 5	0.346 ± 0.019†
rutin	5	0.363 ± 0.033
	10	0.216 ± 0.015
β-carotene + rutin	5 + 5	0.310 ± 0.022†

[a] TBA number expressed as least-squares mean ± SD, $n = 5$. [b] Controls contained no antioxidant agent.
† The TBA number of this combination is significantly lower than that of 10 μM β-carotene at $p < 0.05$.

Table 4. Combined Effect of Vitamin C Plus Phenolic Agent upon Fe^{2+}-Induced Lipid Oxidation in Human Erythrocyte Membrane Ghosts System after a 72-h Incubation at 37 °C

treatment	concn (μM)	TBA number[a]
controls	*b*	0.531 ± 0.032
vitamin C	5	0.475 ± 0.022
	10	0.347 ± 0.033
caffeic acid	5	0.385 ± 0.024
	10	0.215 ± 0.015
vitamin C + caffeic acid	5 + 5	0.161 ± 0.011*
catechin	5	0.265 ± 0.013
	10	0.142 ± 0.012
vitamin C + catechin	5 + 5	0.092 ± 0.010*
myricetin	5	0.464 ± 0.022
	10	0.349 ± 0.034
vitamin C + myricetin	5 + 5	0.267 ± 0.023*
epicatechin	5	0.307 ± 0.018
	10	0.153 ± 0.013
vitamin C + epicatechin	5 + 5	0.084 ± 0.009*
gallic acid	5	0.366 ± 0.025
	10	0.274 ± 0.025
vitamin C + gallic acid	5 + 5	0.141 ± 0.017*
quercetin	5	0.403 ± 0.03
	10	0.289 ± 0.023
vitamin C + quercetin	5 + 5	0.167 ± 0.012*
rutin	5	0.351 ± 0.021
	10	0.209 ± 0.018
vitamin C + rutin	5 + 5	0.175 ± 0.013*

[a] TBA number expressed as least-squares mean ± SD, $n = 5$. [b] Controls contained no antioxidant agent. *The TBA number of this combination is significantly lower than that of 10 μM vitamin C and also lower than that of 10 μM corresponding phenolic agent at $p < 0.05$.

DISCUSSION

The individual antioxidant activity of the seven test phenolic agents has been observed in several different experimental models and animals (Terao et al., 1994; Saija et al., 1995; Nardini et al., 1995). The results of our present study are in agreement with those previous studies and support that these phenolic antioxidants could inhibit Fe^{2+}-induced lipid oxidation in both liposome and human erythrocyte membrane ghost systems (Table 1).

Bors et al. (1990) indicated that the radical scavenging capability of flavonoids was related to three structural groups: (1) the *o*-dihydroxyl structure of the B ring; (2) the 2,3-double bond in conjunction with a 4-oxo function; (3) the additional presence of both 3- and 5-hydroxyl groups. In our present study, quercetin is the only flavonoid that satisfied these three structural characteristics; however, it showed less antioxidant activity than the other three flavonoids (catechin, epicatechin, and rutin) (Table 1). These results suggest that other factors are involved in determining the antioxidant activity of flavonoids. Saija et al. (1995) observed that quercetin showed a greater protective effect than rutin in inhibiting autoxidation of rat cerebral membrane because quercetin displayed a deeper interaction with membranes, while quercetin showed less antioxidant activity than rutin in inhibiting Fe^{2+}-induced linoleate peroxidation. In our present study, Fe^{2+} was used to induce lipid oxidation. The higher partition coefficient of quercetin as compared to that of rutin supported that quercetin was able to interact with biomembranes deeper, which might further result in the lower interaction with water-soluble oxidants such as Fe^{2+}. Consequently, quercetin showed less antioxidant capability than rutin in our two systems (Table 1). The partition coefficient of these five test flavonoids in our present study was negatively correlated with their antioxidant activities in inhibiting Fe^{2+}-induced lipid oxidation in both systems used (Table 1). This also suggests that a flavonoid which interacted with biomembranes deeper displayed less interaction with free radicals present in the aqueous phase. These results support that the partition coefficient of a phenolic antioxidant affects both its interaction with biomembranes and its antioxidant activity performance, particularly when water-soluble oxidants are involved.

A sparing effect of caffeic acid or epicatechin on α-tocopherol has been observed in rat plasma, lipoproteins, and phospholipid bilayers (Terao et al., 1994; Nardini et al., 1997). As shown in Table 2, the combination of α-tocopherol plus gallic acid showed an additive effect, and the combination of α-tocopherol plus caffeic acid, catechin, or epicatechin provided a synergistic effect in delaying Fe^{2+}-induced lipid oxidation. On the basis of the observed synergistic or additive effects in this study, not only caffeic acid and epicatechin but also catechin and gallic acid may possess this sparing effect on α-tocopherol. It is interesting to find that these four phenolic compounds were the most hydrophilic phenolic agents used in this study because of the lower partition coefficients (Table 1). It is likely that a synergistic or additive relationship is present in the two antioxidants with a greater difference in their partition coefficients.

β-Carotene at 10 μM was a weaker antioxidant in this study (Table 1). However, the overall antioxidant activities of β-carotene combined with phenolic antioxidants were greater than β-carotene treatment alone when the total antioxidant concentration was equal (Table 3). These phenolic agents were more hydrophilic than β-carotene (Table 1), and it is interesting to find that the greater antioxidant activity was present in β-carotene combined with caffeic acid, catechin, or epicatechin, the three most hydrophilic phenolic agents used in this study. These results once again suggest that the two antioxidants with a greater difference in their partition coefficients provided greater protection for membrane lipids against oxidation. The effect of β-carotene supplementation alone in preventing oxidation for humans is controversial (Reaven et al., 1993; Oshima et al., 1996). The results of our study suggest that the supplementation of β-carotene with certain phenolic antioxidants might be a better strategy to enhance the antioxidant defense system in humans.

As shown in Table 4, all combinations of vitamin C plus phenolic antioxidants provided synergistic effects. Vitamin C is more hydrophilic than phenolic antioxidants (Table 1). The difference in partition coefficients between vitamin C and phenolic agents might also contribute to the synergistic relationship observed in these combinations. The antioxidant interaction of vitamin C and α-tocopherol has been studied (Yin et al., 1993; Harats et al., 1998). These authors indicated that vitamin C can regenerate α-tocopherol and the combination of these two agents enhanced the oxidation resistance of membrane lipid. The synergistic effect present in the combinations of vitamin C plus seven phenolic antioxidants might also result from the regeneration of phenolic agents from vitamin C. Further study is necessary to demonstrate the regenerative mechanism of these phenolic antioxidants from vitamin C. Since the presence of phenolic antioxidants with vitamin C could enhance the oxidation resistance of human erythrocyte membranes against lipid oxidation, the combined application of these phenolic antioxidants with vitamin C may be more powerful in inhibiting lipid oxidation.

The absorption of phenolic compounds in rats and humans has been studied (Manach et al., 1995; Lee et al., 1995). In rats fed diets containing rutin or quercetin, the concentration of circulating phenolic compounds was $\sim$115 μM (Manach et al., 1995). It is higher than 10 μM, the concentration used in the present study. However, the tea consumption in human subjects resulted in an epigallocatechin concentration in plasma of $\sim$0.6 μM only (van het Hof et al., 1999). Although this concentration is very low, the information regarding the absorption and recovery of other phenolic antioxidants in humans is still inadequate; the study regarding the oral administration of pure phenolic agents in human is lacking. If these phenolic antioxidants can be absorbed and appear in circulation, the intake of these phenolic antioxidants or foods rich in these phenolic antioxidants may not only enhance the human antioxidant defense system directly but also contribute a protective or sparing effect on other nutrients such as α-tocopherol or β-carotene.

In conclusion, partition coefficients of phenolic agents play an important role in determining their individual and combined antioxidant activities with other antioxidants. The greater individual and combined effects of caffeic acid, catechin, and epicatechin support the application of these three phenolic antioxidants with or without α-tocopherol, β-carotene, or vitamin C in human nutrition to prevent or cure oxidation-related diseases

such as cancers, cardiovascular disease, or diabetic complications.

ABBREVIATIONS USED

TCA, trichloroacetic acid; TBA, thiobarbituric acid; PC, phosphatidylcholine; PBS, phosphate-buffered saline.

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