Effects of Dietary Tea Catechins on α-Tocopherol Levels, Lipid Peroxidation, and Erythrocyte Deformability in Rats Fed on High Palm Oil and Perilla Oil Diets

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The effects of dietary tea catechins on the levels of α-tocopherol and lipid peroxidation in both plasma and erythrocytes, as well as their effects on erythrocyte deformability, were examined in rats fed on high palm and perilla oil diets. The decrease in α-tocopherol concentration and the increase in lipid peroxidation level were much more pronounced in the perilla oil group than in the palm oil group. The addition of tea catechins to these diets significantly prevented the α-tocopherol concentration from decreasing. These results suggest that tea catechins may counteract a decrease in α-tocopherol by acting as an antioxidant *in vivo*. Furthermore, the lipid peroxidation in the plasma of rats fed perilla oil was slightly but significantly reduced by the supplemented tea catechins. However, no measurable differences were observed in the deformability of the erythrocytes in any of the groups. It is therefore likely that the erythrocytes are not severely enough affected by the lipid peroxidation to influence their deformability.

Keywords tea catechin; high fat diet; α-tocopherol; lipid peroxidation; erythrocyte deformability

Tea catechins are major constituents of tea, and are known to exhibit, as are various other plant polyphenols, antioxidant activity towards edible oils.[1] Recently, much attention has been focused on the *in vivo* antioxidant activities of these compounds because lipid peroxidation has been reported to be involved in various diseases[2] including liver injury, arterosclerosis, myocardial infarction, inflammation and mutagenicity leading to carbinogenesis. (+)-Catechin ((+)-cyanidanol-3), which is a minor component of tea catechin, has been found to have a protective action against a number of hepatotoxic agents both *in vitro* and *in vivo*.[3] This protective action is believed to be due to the inhibition of lipid peroxidation in the liver. Okuda *et al.*[4] have reported that tea catechins such as (−)-epicatechin, (+)-catechin and (−)-epigallocatechin gallate, inhibit lipid peroxidation in rat liver mitochondria stimulted by ADP and ascorbic acid, as well as lipid peroxidation in rat liver microsomes stimulated by ADP and NADPH. It has also been shown that tea catechins terminate autooxidation of fatty acids by functioning as radical scavengers.[5] Furthermore, liver injury and the accumulation of lipid peroxides in the serum of rats fed on peroxidized oil have been found to be suppressed by the oral administration of catechin fractions extracted from various kinds of tea.[6]

It is well known that polyunsaturated fats have many beneficial effects such as hypolipidemic activity, suppression of platelet aggregability and tumorigenesis, and an increase in mean survival time.[7] In addition, lipid peroxidation *in vivo* has been found to be accelerated by dietary polyunsaturated fats, particularly under conditions of vitamin E-insufficiency[8]; also the lipid peroxidation may or may not depend on the degree of unsaturation.[9] In addition, the possibility that such lipid peroxidation affects the deformability of erythrocytes can not be excluded, given that the polyunsaturated fatty acids and oxygen in red cells makes them susceptible to lipid peroxidation.[10]

In the present paper, we describe, in rats fed on high palm oil and perilla oil diets, the effects of dietary tea catechins on the levels of α-tocopherol and lipid peroxides in both plasma and erythrocytes. Furthermore, the influence of these catechins on erythrocyte deformability is examined.

Materials and Methods

Materials Tea catechins of 90% purity were prepared from green tea leaves according to the method previously reported.[11] The catechins consist of (+)-catechin (1.4%), (−)-epicatechin (5.8%), (−)-epigallocatechin (17.6%), (−)-epicatechin gallate (12.5%), (−)-epigallocatechin gallate (53.9%), and others (9.8%).

Animals and Diets Male Wistar rats (Charles River), 5 weeks of age (115—135 g), were divided into 4 groups of the same average weight. Over a period of 31 d, each group of six rats was fed one of the following 4 diets: 1) 30% palm oil diet; 2) 30% palm diet containing 1% tea catechins; 3) 30% perilla oil diet; 4) 30% perilla oil diet containing 1% tea catechins. Tables I and II show the diet compositions and the fatty acid compositions of the oils, respectively. α-Tocopherol was added to give a final concentration of 6 mg/100 g in each diet, taking into account the content of α-tocopherol already present in the palm and perilla oils. Diets with peroxidative values below 10 meq/kg were used throughout the experiment. The animals were housed individually in cages. Room temperature was

TABLE I. Composition of High Fat Diets

Ingredient	Palm oil group		Perilla oil group	
	Control	Catechin	Control	Catechin
Corn starch (%)	28.9	28.9	28.9	28.9
Sucrose (%)	10.0	10.0	10.0	10.0
Casein (%)	20.0	20.0	20.0	20.0
Palm oil (%)	30.0	30.0	—	—
Perilla oil (%)	—	—	30.0	30.0
Cellulose (%)	5.0	5.0	5.0	5.0
Salt mixture[a] (%)	4.0	4.0	4.0	4.0
Choline chloride (%)	0.1	0.1	0.1	0.1
Vitamin mixture[a] (%) (vitamin E free)	2.0	2.0	2.0	2.0
Tea catechin (%)	—	1.0	—	1.0
α-Tocopherol[b] (mg)	3.7	3.7	4.3	4.3

a) Salt mixture and vitamin mixture (vitamin E free) according to Harper were purchased from Oriental Kobo Kogyo Co. *b*) Taking into account the content of α-tocopherol in the palm and perilla oils, the final concentration of the tocopherol in the diets was adjusted to 6 mg/100 g.

controlled at 23±2 °C and the room was on a 12 h light–dark cycle. Fresh food and water were available *ad libitum*.

Analytical Method Rats were fasted overnight and anesthetized with ether. Blood samples were collected in heparinized syringes by means of heart puncture and instantly chilled in an ice-water bath. The blood was then centifuged at 1800×*g* for 10 min at 4 °C. The plasma and red cells were separated in order to determine both α-tocopherol and lipid peroxidation levels. The plasma lipid concetration was measured by enzyme assay (Wako kit). α-Tocopherol in the plasma and erythrocytes was extracted by the method of Mino *et al.*[12] and the concentrations were then determined by HPLC analysis. HPLC was performed using a Capcellpak C18 column (4.6×250 mm) installed in a Shimadzu liquid chromatograph equipped with a UV spectrophotometric detector and a Shimadzu C-R3A integrator. Elution was carried out using methanol at a flow rate of 1.0 ml/min. The elution pattern was monitored by measuring the absorbance at 280 nm. Lipid peroxidation was monitored by measuring the production of malondialdehyde using the thiobarbituric acid (TBA) assay. TBA reactants in the plasma were determined by the method of Buege and Aust.[13] The white ghosts of red cells were prepared by the method of Tomoda *et al.*[14] After incubation of the ghost (0.6—1.0 mg protein/190 μl) with 10 μl of 24 mM *tert*-butyl hydroperoxide or water for 30 min at 37 °C, TBA reactants were determined as described above. The protein concentration of the ghosts was determined by the method of Gotham *et al.*[15] The hematological properties of the erythrocytes were measured at Shida Medical Analysis Center (Fujieda, Japan). The osmotic fragility of the erythrocytes was determined by the method described by Eskelinen and Saukko.[16]

Erythrocyte Deformability After centrifugation of the blood samples, plasma and erythrocytes were separated, taking care that there was no contamination by white cells. A suspension of erythrocytes with a hematocrit of approximately 20% was then prepared using the plasma or phosphate-buffered saline (pH 7.4) containing 1.0% bovine serum albumin. The hematocrits of the samples prepared were than determined exactly using a microhematocrit centrifuge (11000×*g*, 5 min). The deformability of the erythrocyte plasma or erythrocyte buffer suspension was measured using apparatus equipped with a new design of silicon filter[7] instead of the Nuclepore filter generally used. This silicon filter has 2600 microchannels (equivalent diameter 5 μm and length 14 μm). The actual apparatus and procedures were the same as those used in the modified Nuclepore filtration method developed by Kikuchi *et al.*[18] The samples were examined using the above apparatus and the time taken for 100 μl of the erythrocyte suspension to pass through the microchannels was determined under a negative pressure of 20 cm water. The transit time of the erythrocyte suspension was taken as an indication of erythrocyte deformability.

Statistical Analysis The significance of the differences between the groups tested was determined by Student's *t* test. Differences were considered to be statistically significant when $p<0.05$.

Results and Discussion

There were no significant differences among the groups tested in terms of either body weight gain or food intake. The plasma lipid levels in the perilla oil fed rats were markedly lower than those in the palm oil fed rats, regardless of tea catechin supplementation (Table III). It is well recognized that polyunsaturated fats in the diet decrease serum cholesterol and triglyceride concentration[8a,19] and our results were consistent with these previous findings.

We first investigated in the rats fed on palm oil and perilla oil diets, the effects of dietary tea catechins on the levels of both α-tocopherol and TBA-reactive substances in the plasma and erythrocytes. Results are given in Table IV. Plasma tocopherol concentrations were higher in the palm oil group than in the perilla oil group. The feeding of tea catechins to both groups significantly prevented a decrease in the tocopherol levels. The change in tocopherol concentration in the erythrocytes was similar to that in plasma. Thus, the tocopherol concentration in the erythrocytes appeared to be related to the plasma concentration. These results suggest that tea catechins may counteract a decrease in plasma α-tocopherol by functioning as an antioxidant *in vivo*, although it is unclear whether the α-tocopherol is directly protected by the catechins or not.

The level of TBA-reactive substances in the plasma was higher in the rats fed perilla oil than that in the rats fed palm oil. The addition of tea catechins was found to be effective in suppressing the lipid peroxidation levels in the perilla oil group. These observations suggest that the lipid peroxidation levels in the plasma are closely related to the plasma α-tocopherol concentration. Thus, it appears that

Table II. Fatty Acid Composition of Palm and Perilla Oils

Fatty acid	Palm oil (%)	Perilla oil (%)
14:0	1.1	—
16:0	46.6	6.7
16:1	—	—
18:0	3.8	2.1
18:1	37.5	17.7
18:2	9.8	15.5
18:3	—	52.6
20:0	0.2	—

Table III. Concentration of Cholesterol, Phospholipids, and Triglycerides in Plasma

Lipids	Palm oil group		Perilla oil group	
	Control	Catechin	Control	Catechin
Cholesterol (mg/dl)	65.9± 2.7	66.7±10.0	36.8± 2.9[a]	24.7± 5.0[a,b]
Phospholipids (mg/dl)	126.9±11.4	121.1± 9.5	68.0±14.9[a]	61.7±11.1[a]
Triglycerides (mg/dl)	80.3±10.7	67.3±16.3	32.2± 8.0[a]	26.3± 3.7[a]

Values were expressed as mean±S.D. *a*) Statistically significant differences compared with palm oil group, $p<0.01$. *b*) Statistically significant differences compared with perilla oil group, $p<0.001$.

Table IV. Levels of α-Tocopherol and TBA-Reactive Substances in Plasma and Erythrocytes

	α-Tocopherol		TBA-reactive substances		
	Plasma (μg/dl)	Erythrocyte (μg/dl packed cells)	Plasma (nmol/ml)	Erythrocyte (nmol/mg protein)	Erythrocyte treated by BHP (nmol/mg protein)
Palm oil	433.9±22.8	122.3±11.4	1.18±0.15	0.48±0.04	1.18±0.10
Palm oil+catechin	493.4±34.6[a]	172.5±21.4[c]	1.16±0.29	0.52±0.08	1.28±0.14
Perilla oil	55.2± 8.1[c]	46.4± 2.6[c]	3.86±0.25[c]	0.64±0.03[c]	2.08±0.21[c]
Perilla oil+catechin	134.2±26.9[c,f]	102.9±39.1[c,e]	3.39±0.28[c,d]	0.63±0.05[c]	1.75±0.37[c]

Values were expressed as mean±S.D. Statistically significant differences compared with palm oil group; *a*) $p<0.05$, *b*) $p<0.01$, and *c*) $p<0.001$. Statistically significant differences compared with perilla oil group; *d*) $p<0.05$, *e*) $p<0.01$, and *f*) $p<0.001$. Abbreviation: BHP, *tert*-butyl hydroperoxide.

TABLE V. Hematological Properties of Erythrocytes

	Palm oil group		Perilla oil group	
	Control	Catechin	Control	Catechin
Hematocrit (%)	42.88±1.24	41.75± 1.70	41.30± 1.98	42.50±1.43
Erythrocyte (10^5/mm^3)	73.93±2.95	72.10± 3.79	73.93± 3.39	74.25±1.97
MCV (μ^3)	58.17±1.47	57.83± 2.14	56.67± 0.82	57.33±1.03
MCH (pg)	19.85±0.26	19.80± 0.58	19.22± 0.26	19.32±0.47
MCHC (%)	34.20±0.33	32.20± 0.50	34.45± 0.58	33.58±0.62
Hemolysis (% in 0.3% NaCl)	72.38±9.39	55.87±14.54[a)]	80.66±12.04	83.97±9.06

a) Statistically significant differences compared with the control in palm oil group, $p<0.05$.

tea catechins may counteract an increase in lipid peroxidation in the plasma by maintaining α-tocopherol levels. Tea catechins had little effect on lipid peroxidation in the erythrocytes, although the α-tocopherol levels in the catechin-supplemented groups were higher than those in the groups not receiving catechins. Furthermore, only a slight difference in TBA-reactive substances between the two groups was observed. These results imply that protection of the erythrocyte membrane from lipid peroxidation may be dependent not only on α-tocopherol but also on such enzyme factors as superoxide dismutase, catalase and peroxidase. However, differences in the erythrocyte TBA-reactive substances between the palm oil and the perilla oil groups were pronounced when the erythrocytes were treated with *tert*-butyl hydroperoxide. It appears that the fatty acid composition of the erythrocyte membrane may depend on the type of dietary fat, as reported by Sakai *et al.*[20] and Rao *et al.*[21]

The effects of tea catechins on the hematological properties and fragility of the erythrocytes were also examined (Table V). There were no differences in hematocrit, red blood cell number (RBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), or mean corpuscular hemoglobin concentration (MCHC) among the 4 dietary groups. The osmotic fragility was significantly changed by tea catechins in the palm oil fed rats. However, there is no reasonable explanation for the alteration of erythrocyte fragility produced by the catechins in the palm oil fed rats, although osmotic fragility is a commonly used criterion to determine the strength or stability of the erythrocyte membrane.

The effects of tea catechins on erythrocyte deformability were further examined. In preliminary experiments, we found that the shape of rat erythrocytes changes instantly from discocytes to echinocytes at room temperature, even in the case of erythrocytes present in the plasma. This alteration in cell shape causes decreased deformability. Protection of the red cell shape was achieved by chilling the erythrocytes to below 4 °C or adding bovine serum albumin. The preparation of erythrocyte plasma and buffer suspension was carried out at 4 °C and the samples were kept at this same temperature until measurements were carried out. Figure 1 shows the transit time through the microchannels of erythrocyte–plasma and erythrocyte–buffer suspensions. Despite the differences in α-tocopherol levels, lipid peroxidation and osmotic fragilities described above, no measurable differences were detected in erythrocyte deformability among the four groups. In addition, the

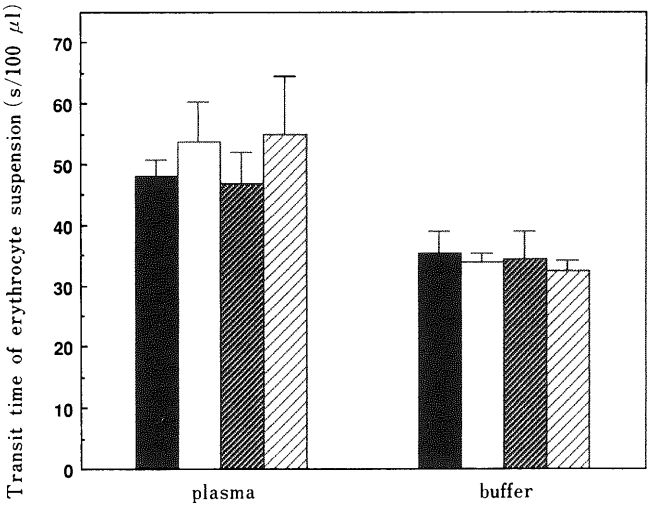

Fig. 1. Effects of Tea Catechins on Deformability of Erythrocytes

■, erythrocytes from rats fed palm oil; □, erythrocytes from rats fed palm oil + tea catechins; ▨, erythrocytes from rats fed perilla oil; ▨, erythrocytes from rats fed perilla oil + tea catechins.

transit time of the erythrocytes observed in these four groups was confirmed to be almost the same as that of rats fed on a normal diet (data not shown). It is likely that the changes in lipid peroxidation, α-tocopherol levels, or osmotic fragility in the erythrocytes were not extreme enough to alter their deformability. Thus, in this case it was observed that neither the supplementation of different types of fats nor tea catechins affected erythrocyte deformability.

Our investigations confirmed that the levels of α-tocopherol and lipid peroxidation in plasma and erythrocytes were affected significantly by the type of dietary fat. The addition of tea catechins was found to prevent a decrease in plasma and erythrocyte α-tocopherol levels. These results suggest that tea catechins are capable of acting as an *in vivo* antioxidant. Lipid peroxidation was also found to be suppressed by catechins, especially in the perilla oil group. This observation may be closely linked to the action of tea catchins in preventing α-tocopherol destruction. The deformability of the erythrocytes was not affected either by the type of dietary fat or by supplemented catechins.

The tea catechin dosage used in this study is considered to be equivalent to a dosage of 4.5 g/d for humans. While this intake of tea catechins could be regarded as excessive with respect to daily tea drinking, from a pharmacological point of view it could be easily achieved as a drug. Previously, large amounts of (+)-catechin (1.5—3.0 g) administered to patients with chronic hepatitis were reported to be effective in increasing the level of vitamin E and decreasing TBA-reactive substances in blood.[3e] Further studies are necessary to examine whether tea catechins have any beneficial effects at lower dosages or over longer periods of administration.

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