Comparison of the Protective Effects of Epigallocatechin Gallate and Epigallocatechin on Paraquat-Induced Oxidative Stress in Rats

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The protective effects of (−)-epigallocatechin gallate (EGCg) and (−)-epigallocatechin (EGC) on paraquat-induced oxidative stress were compared in rats. Both EGCg and EGC effectively relieved decreases in food intake and body weight gain which were a result of administering the paraquat diet. The concentration of thiobarbituric acid-reactive substances (TBARS) in the liver of rats fed with the EGCg-added paraquat diet was lower than that of the EGC-added paraquat diet. The antioxidative enzyme activities in serum and liver, except for the catalase activity of the liver mitochondrial fraction, were not significantly different among the control, paraquat, paraquat+ EGCg, and paraquat+EGC diet-fed rats. The catalase activity of the liver mitochondrial fraction was markedly decreased by feeding the paraquat diets and the decrease was strongly relieved by supplementing EGCg rather than EGC in the paraquat diet. On the other hand, decreases in the liver glutathione, cysteine and triacylglycerol concentrations that were caused by the paraquat diet had greater relief by supplementing EGCg rather than EGC in the paraquat diet. These results may suggest that the gallic acid moiety of the EGCg molecule plays an important role in demonstrating stronger protective effects for paraquat-induced oxidative stress.

Keywords: paraquat, epigallocatechin gallate, epigallocatechin, thiobarbituric acid reactive substances, antioxidative enzyme activity

Recently, phenolic compounds which are contained in many food products such as fruits and vegetables received much attention because of their antioxidative activities *in vitro* and *in vivo* (Shiraki & Hara, 1990; Hara, 1994; Yoshino *et al.*, 1994; Chen & Ho, 1995), and their antimutagenic (Kada *et al.*, 1985; Yen & Chen, 1995), antitumor (Yoshizawa *et al.*, 1987; Hara *et al.*, 1989; Susanne *et al.*, 1996), antibacterial (Toda *et al.*, 1991) and hypocholesterolemic (Fukuyo *et al.*, 1986) activities. Of all phenolic compounds, catechins which are contained as major phenolic compounds in green tea, have been especially focussed on by many people because of their physiological functions, and because they have been consumed in high quantities in the form of green tea in Japan and China. It has recently been pointed out that coronary heart disease is closely related to the oxidation of low density lipoprotein (LDL) (Frankel *et al.*, 1993) and that catechins in red wine are effective in preventing the oxidation of LDL (Bell & White, 1992; Teissedre *et al.*, 1996). On the other hand, we have previously observed that (−)-epigallocatechin gallate (EGCg) relieved paraquat-induced oxidative stress in rats, suggesting that the other catechins in green tea may also be effective in reducing oxidative stress by paraquat (Suzuki *et al.*, 1997). However, the relation between the chemical structures of catechins and their physiological activities in inhibiting the oxidation *in vivo* have not yet been fully examined.

This paper investigates whether (−)-epigallocatechin (EGC), which lacks the gallic acid moiety in the EGCg molecule, also has a protective effect against paraquat-induced oxidative stress, and further whether the protective effects of EGCg and EGC differ depending on the chemical structures of EGCg and EGC.

Material and Methods

Catechins and paraquat EGCg and EGC were prepared from green tea by Mitui Norin Co., Ltd. (Fujieda, Shizuoka). The purity level of each EGCg and EGC was more than 95% when analyzed by HPLC using an ODS column and a mixture of acetonitrile-ethyl acetate-0.5% phosphoric acid=12-2-86 (v/v) as a developing solvent. Paraquat (methyl viologen) was purchased from Sigma Chemical Co., Ltd. (St. Louis, Mo).

Animals and diets Five-week-old Wistar strain male rats (Japan SLC, Hamamatsu), each weighing about 61 g, were randomly divided into 5 groups of 5–6 rats each. The rats were individually housed in stainless-steel cages with screen bottoms and kept under control conditions with a 12-h light and 12-h dark cycle (06:00–18:00 light), a temperature range of 22–24°C, and a relative humidity level of about 55%.

The composition of the experimental diets is given in Table 1. There were five kinds of diets: One the basal diet without paraquat (20C), and four paraquat diets with or without

Table 1. Composition of the diets (%).

Ingredient	Diet				
	20C[a]	20C+PQ[b]	20C+PQ+0.1% EGCg[c]	20C+PQ+ 0.063% EGC[d]	20C+PQ+ 0.032% EGC[e]
Casein	20.0	20.0	20.0	20.0	20.0
α-Corn starch : Sucrose (1 : 1)	65.5	65.48	65.38	65.417	65.448
Cellulose	5.0	5.0	5.0	5.0	5.0
Corn oil	5.0	5.0	5.0	5.0	5.0
Mineral mixture[f]	3.5	3.5	3.5	3.5	3.5
Vitamin mixture[g]	1.0	1.0	1.0	1.0	1.0
Paraquat	—	0.02	0.02	0.02	0.02
EGCg	—	—	0.1	—	—
EGC	—	—	—	0.063	0.032

[a]20C, 20% casein diet; [b]20C+PQ, 20% casein diet with 0.02% paraquat; [c]20C+PQ+0.1% EGCg, 20% casein diet with both 0.02% paraquat and 0.1% EGCg; [d]20C+PQ+0.063% EGC, 20% casein diet with both 0.02% paraquat and 0.063% EGC; [e]20C+PQ+0.032% EGC, 20% casein diet with both 0.02% paraquat and 0.032% EGC. EGCg, Epigallocatechin gallate; EGC, epigallocatechin. [f]AIN-93G-MX and [g]AIN-93-VX were obtained from Oriental Yeast Co., Tokyo.

catechins (20C+PQ, 20C diet with 0.02% paraquat; 20C+PQ+0.1% EGCg, 20C diet with both 0.02% paraquat and 0.1% EGCg; 20C+PQ+0.063% EGC, 20C diet with both 0.02% paraquat and 0.063% EGC; 20C+PQ+0.032% EGC, 20C with both 0.02% paraquat and 0.032% EGC). The amount of 0.063% EGC was equal to that of 0.1% EGCg on a molar basis. EGCg and EGC were added to the diets at the expense of the mixture of α-corn starch and sucrose (1 : 1). Food and water were provided *ad libitum* for 10 days.

Collection and preparation of blood and liver Blood was collected by heart puncture from rats that had been anesthetized with Nembutal (Dainippon Pharmaceutical Co., Osaka) 10 h after starvation at the end of the feeding period. An aliquot of 0.1 ml of the blood was added to 1.9 ml of physiological saline by gently shaking, and the mixture was then centrifuged at 1000×*g* for 10 min in order to obtain a serum for measuring blood TBARS levels as well as to provide erythrocytes. The erythrocytes were lysed with H_2O in order to measure the antioxidative enzyme activities. A section of the right lobe in the liver which had been excised from each of the rats was used to measure liver superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase, and glutathione reductase (GSSG-R) activities. The other parts of the liver were stored at −28°C until needed for measuring lipid and TBARS concentrations. Centrifuging the blood at 1000×*g* for 15 min separated the serum used for measuring the lipids.

Preparation of subcellular fractions A supernatant for measuring antioxidative enzyme activities in the liver was prepared according to the method of Del Boccio *et al.* (1990). One gram of the right lobe was homogenized in 5 ml of ice-cold 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA in a homogenizer with a teflon pestle, and the homogenate was then mixed with 2 volumes of 2.3% KCl, followed by centrifugation at 600×*g* for 3 min at 4°C to remove fibrous material. The supernatants which were obtained were further centrifuged at 1400×*g* for 10 min at 4°C to remove cellular debris. An aliquot of the supernatant was subsequently sonicated at 30 W for 2 min (4 times for 30 s) in a Bransonic model 1200 sonicator (Yamato Co., Tokyo). It was then centrifuged at 10,000×*g* for 30 min at 4°C to obtain the mitochondrial fraction for measuring SOD and catalase

activities. An aliquot of the supernatant was centrifuged at 105,000×*g* for 60 min at 4°C to obtain the cytosolic fraction for measuring SOD, catalase, GSH-Px and GSSG-R activities.

Measurements of enzyme activities SOD activity was measured by xanthine-xanthine oxidase-nitroblue tetrazolium (NBT) system (Imanari *et al.*, 1977), and the catalase activity was measured by the spectrophotometric method, following a decrease in absorbance at 240 nm at 25°C, due to hydrogen peroxide decomposition (Tomita & Sano, 1983). GSH-Px activity was measured by the procedure of Lawrence and Burk (1976), using *t*-butyl hydroperoxide as a substrate and following the decrease in absorbance of NADPH at 340 nm (He & Yasumoto, 1990). The definition of one unit of SOD and catalase activities is the amount of enzyme required to inhibit the rate of diformazan formation from NBT by 50% per mg of hemoglobin or protein, and the amount of enzyme to decompose 1 μmol of H_2O_2 in min per mg of hemoglobin or protein, respectively. The definition of one unit of GSH-Px and GSSG-R activities is the amount of enzyme that is required to oxidize 1 μmol of NADPH per mg of hemoglobin or protein.

The hemoglobin and protein contents were measured using a commercial hemoglobin test kit (Wako Pure Chemical Ind., Osaka) and by the method of Lowry *et al.* (1951), respectively.

Measurement of TBARS Serum TBARS concentration was determined by the method of Yagi (1976) and is expressed as nmol of malondialdehyde per ml of blood. Liver TBARS concentration was measured by the method of Uchiyama and Mihara (1978) using the homogenate which had been obtained by homogenizing 1 g of frozen rat liver (a section from the main lobe) with 9 ml of 1.15% KCl.

Lipid analyses The total cholesterol, triacylglycerol, and phospholipid in the serum were enzymatically measured using commercial kits (cholesterol E- test, triglyceride E-test, and phospholipid B-test, respectively; Wako Pure Chemical Ind.). Serum HDL-cholesterol was enzymatically measured in the supernatant obtained after heparin-Mn precipitation of the other lipoproteins (Burstein *et al.*, 1970).

The lipids of a frozen liver were extracted and purified by the method of Folch *et al.* (1957). The lipids in the extract

were measured using the same kits as those in the case of the serum with the exception that a Mono-test cholesterol (Boehringer Manheim Yamanouchi Co., Tokyo) was used instead of the cholesterol E-test.

Measurement of reduced glutathione and cysteine The liver (0.5 g) which had been frozen after excising from the rats was homogenized with 5 ml of 0.034 M EDTA and 5 ml of 30% metaphosphoric acid cooling in ice as described by Takahashi *et al.* (1979). A 0.1 ml portion of the supernatant obtained by centrifuging the homogenate at $2500 \times g$ for 15 min at 4°C was mixed with 10 μl of 5 N NaOH, 300 μl of 0.5 M borate buffer (pH 8.8), 20 μl of 0.02 M EDTA, and 50 μl of 3 nM N-(9-acridinyl) maleimide (NAM) and was then followed by reaction for 3 h at 20°C. Analytical conditions in HPLC were the same as that described by Takahashi *et al.* (1979), except that a column of Develosil ODS-HG-5 (4.6× 150 mm, Nomura Chemical Co., Ltd., Seto-shi) was used.

Analyses of lung The lungs which were excised from each rat immediately after the collection of blood and liver were stored at −28°C until TBARS and antioxidative enzyme activities could be determined. To determine lung SOD, catalase and GSH-Px activities, 1 g of the frozen sample was homogenized with 5 ml of 0.2% Triton X-100 (v/v) at 4°C in a glass-glass homogenizer (Paynter *et al.*, 1979) and then centrifuged at $10,000 \times g$ for 30 min at 4°C. An aliquot of the supernatant was mixed with 0.25 volume of ethyl alcohol and 0.15 volume of chloroform, before being centrifuged at $5000 \times g$ for 20 min at 4°C. The supernatant obtained was used for measuring enzyme activities.

Statistical analyses The data for each of the 5 groups were statistically analyzed by Duncan's multiple range test after one-way analysis of variance (ANOVA) with the exception that food intake and body weight after 9 days were compared using the Mann-Whitney test for their nonparametric data. Significant differences in the means were inspected at $p < 0.05$.

Results

Food intake and body weight gain As shown in Fig. 1, food intake and body weight showed a tendency to
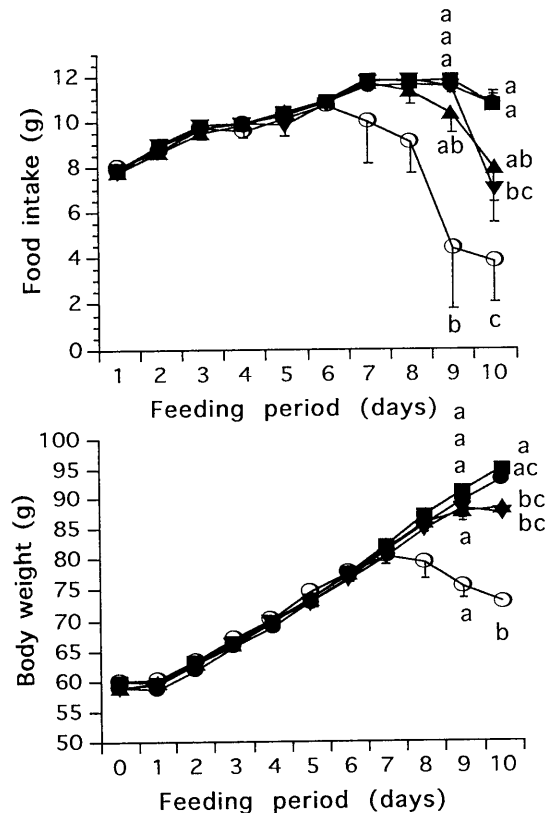

Fig. 1. Effects of the dietary epigallocatechin gallate and epigallocatechin on the food intake and on body weight gain of rats fed on the paraquat diet. ●, 20C group; ○, 20C+PQ group; ■, 20C+PQ+0.1% EGCg group; ▲, 20C+PQ+0.063% EGC group; ▼, 20C+PQ+0.032% EGC group. Each symbol is expressed as mean±SE. Values not sharing a common letter are significantly different at $p < 0.05$ within each indicated day.

Table 2. Effects of EGCg and EGC on the serum and liver TBARS levels, and on the erythrocytes and liver antioxidative enzyme activities of rats induced oxidative stress by paraquat.

Diet	20C	20C+PQ	20C+PQ+ 0.1% EGCg	20C+PQ+ 0.063% EGC	20C+PQ+ 0.037% EGC
Initial body weight (g)	59±1[a]	60±1[a]	60±1[a]	59±1[a]	59±1[a]
Liver weight (g/100 g of body weight)	3.6±0.1[a]	3.8±0.1[a]	3.8±0.2[a]	3.9±0.2[a]	3.7±0.1[a]
Serum TBARS (nmol/ml of blood)	0.56±0.13[a]	0.54±0.30[a]	0.36±0.08[a]	0.25±0.09[a]	0.47±0.12[a]
Liver TBARS (nmol/mg of total lipid)	2.79±0.08[bc]	3.91±0.27[a]	2.60±0.17[bc]	2.71±0.37[a]	3.33±0.07[a]
Antioxidative enzyme activitiy in erythrocytes					
SOD (U/mg of Hb)	1.86±0.09[a]	1.50±0.13[a]	1.83±0.10[a]	1.63±0.23[a]	1.68±0.12[a]
Catalase (U/mg of Hb)	61.3±6.3[a]	62.9±1.5[a]	69.7±5.4[a]	55.5±7.3[a]	62.7±2.4[a]
GSH-Px (U/mg of Hb)	0.060±0.009[a]	0.069±0.009[a]	0.077±0.013[a]	0.097±0.019[a]	0.073±0.004[a]
in liver cytosol fraction					
SOD (U/mg of protein)	2.36±0.06[a]	2.52±0.10[a]	2.44±0.07[a]	2.54±0.07[a]	2.61±0.05[a]
Catalase (U/mg of protein)	278±30[a]	323±19[a]	281±38[a]	242±19[a]	253±18[a]
GSH-Px (U/mg of protein)	0.22±0.01[a]	0.24±0.01[a]	0.24±0.02[a]	0.24±0.01[a]	0.22±0.01[a]
GSSG-R (U×10⁻²/mg of protein)	1.18±0.04[a]	1.31±0.09[a]	1.17±0.08[a]	1.24±0.05[a]	1.20±0.02[a]
in liver mitochondrial fraction					
SOD (U/mg of protein)	5.22±0.61[a]	3.69±0.76[a]	4.62±0.62[a]	5.08±0.92[a]	4.88±0.64[a]
Catalase (U/mg of protein)	562±84[a]	203±26[b]	579±87[a]	306±125[b]	314±86[b]

Values are the means±SE for 5–6 rats per group. Mean values within the same row that are not followed by a common superscript letter are significantly different as assessed by analysis of variance (ANOVA) and Duncan's multiple-range test ($p < 0.05$).

decrease after 7 and 8 days in the rats fed a paraquat diet (20C+PQ group) compared with the figures in the control rats. The supplements of EGCg and EGC in the paraquat diet relieved the decreases in both food intake and body weight.

TBARS and antioxidative enzyme activities As shown in Table 2, although the serum TBARS concentration in the paraquat-fed rats did not differ from that of the control rats, supplements of EGCg and EGC in the paraquat diet had a tendency to cause a decrease in the TBARS concentration compared to that of the paraquat-fed rats. A significant increase was found in the liver TBARS concentration of the paraquat-fed rats compared with that of the control rats, and the increased value returned to the same level of the control by supplementing EGCg in the paraquat diet. Supplementing EGC in the paraquat diet had a tendency to relieve the increase in TBARS concentration in the liver from the paraquat diet but not to a statistically significant degree.

The erythrocyte SOD, catalase and GSH-Px activities were not so different among the control, paraquat-fed, PQ+ EGCg-fed, and PQ+EGC-fed rats (Table 2). The catalase activity in the liver cytosol fraction showed a tendency to increase in the paraquat-fed rats, compared to that of the control rats. By supplementing EGCg and EGC in the

paraquat diet, this increase tended to be relieved. In contrast, the catalase activities in the mitochondrial fraction markedly decreased in the paraquat-fed rats, compared to that of the control rats. The supplements of EGCg and EGC in the paraquat diet relieved significantly and seemed to relieve this decrease, respectively. The SOD, GSH-Px, and GSSG-R activities in the liver cytosol fraction and the SOD activity in the mitochondrial fraction did not show any statistically significant differences among the 5 groups.

Lipid levels As shown in Table 3, the serum total cholesterol, HDL-cholesterol, triacylglycerol, and phospholipid concentrations were not statistically affected by the addition of paraquat to the control diet or by supplementing EGCg and EGC in the paraquat diet. However, the atherogenic index had a tendency to increase in the rats fed the paraquat diet, compared to the control rats, and this increase tended to return to the control rat's level when EGCg and EGC were added to the paraquat diet.

The liver triacylglycerol concentration decreased markedly when the rats were fed the paraquat diet. The values that were decreased returned to a level higher than the control rat's level and tended to return to the control rat's level when EGCg and EGC were supplemented in the paraquat diet, respectively.

Table 3. Effects of EGCg and EGC on the serum and liver lipid concentrations.

Diet	20C	20C+PQ	20C+PQ+ 0.1% EGCg	20C+PQ+ 0.063% EGC	20C+PQ+ 0.037% EGC
in Serum					
Total cholesterol (mmol/l)	1.89±0.16[a]	2.04±0.19[a]	2.02±0.08[a]	1.78±0.13[a]	1.90±0.07[a]
HDL-cholesterol (mmol/l)	1.33±0.03[a]	1.37±0.08[a]	1.50±0.05[a]	1.31±0.09[a]	1.35±0.09[a]
Triacylglycerol (mmol/l)	0.42±0.05[a]	0.36±0.05[a]	0.48±0.05[a]	0.50±0.10[a]	0.40±0.06[a]
Phospholipid (mmol/l)	1.51±0.03[a]	1.49±0.16[a]	1.67±0.05[a]	1.50±0.13[a]	1.43±0.07[a]
Atherogenic index[1]	0.41±0.02[a]	0.59±0.22[a]	0.34±0.02[a]	0.39±0.04[a]	0.42±0.05[a]
in Liver					
Total cholesterol (μmol/g of liver)	10.0±0.1[ab]	8.36±0.31[b]	10.0±0.2[a]	8.77±0.35[b]	8.18±0.26[b]
Triacylglycerol (μmol/g of liver)	19.6±1.3[b]	4.8±0.7[d]	25.3±2.8[a]	11.2±1.9[c]	6.2±0.9[cd]
Phospholipid (μmol/g of liver)	15.6±0.4[a]	15.1±0.1[a]	16.5±0.6[a]	15.7±0.9[a]	14.8±0.3[a]

Values are the means±SE for 5–6 rats per group. Mean values within the same row that are not followed by a common superscript letter are significantly different as assessed by analysis of variance (ANOVA) and Duncan's multiple-range test ($p<0.05$). [1](Total cholesterol−HDL-cholesterol)/HDL-cholesterol (i.e. (VLDL-cholesterol+LDL-cholesterol)/HDL-cholesterol)).

Table 4. Effects of EGCg and EGC on the liver GSH and Cys contents in Paraquat-fed rats.

Diet	20C	20C+PQ	20C+PQ+ 0.1% EGCg	20C+PQ+ 0.063% EGC	20C+PQ+ 0.037% EGC
GSH (μmol×10^{-2}/g of liver)	5.62±0.28[a]	3.63±0.14[c]	5.25±0.38[a]	4.40±0.25[b]	4.42±0.16[b]
Cys (μmol/g of liver)	0.68±0.06[a]	0.26±0.10[c]	0.70±0.10[a]	0.38±0.05[bc]	0.59±0.10[ab]

GSH, glutathione; Cys, cysteine. Mean values within the same row that are not followed by a common superscript letter are significantly different as assessed by analysis of variance (ANOVA) and Duncan's multiple-range test ($p<0.05$).

Table 5. Effects of EGCg and EGC on the lung TBARS and antioxidative enzyme activities.

Diet	20C	20C+PQ	20C+PQ+ 0.1% EGCg	20C+PQ+ 0.063% EGC	20C+PQ+ 0.037% EGC
TBARS (nmol/g of lung)	74.8±0.8[a]	71.4±10.4[a]	70.9±2.1[a]	71.0±7.9[a]	79.3±6.9[a]
SOD (U/mg of protein)	2.40±0.03[a]	2.53±0.15[a]	2.50±0.09[a]	2.47±0.06[a]	2.56±0.15[a]
Catalase (U/mg of protein)	152±4[bc]	139±11[c]	171±3[ab]	167±6[ab]	172±13[a]
GSH-Px (U/mg of protein)	0.56±0.04[a]	0.55±0.02[a]	0.53±0.02[a]	0.55±0.01[a]	0.52±0.02[a]

Values are the means±SE for 5–6 rats per group. Mean values within the same row that are not followed by a common superscript letter are significantly different as assessed by analysis of variance (ANOVA) and Duncan's multiple-range test ($p<0.05$).

The magnitude of EGCg needed to suppress the decrease in the triacylglycerol was greater than that of EGC. The liver total cholesterol revealed a tendency to decrease in the paraquat-fed rats, and the decrease was relieved by supplementing EGCg in the paraquat diet. However, the effect of EGC in relieving the decrease was weaker than that of EGCg.

Glutathione and cysteine The results are shown in Table 4. Reduced glutathione and cysteine concentrations in the paraquat-fed rats were significantly lower than those of the control rats. The supplement of EGCg in the paraquat diet relieved these decreases, but the magnitude of EGC needed to relieve the decreases was smaller than that for EGCg.

Lung TBARS and antioxidative enzyme activities There were no statistically significant differences in TBARS concentrations among the rats fed the control and paraquat diets, or the paraquat diets supplemented with EGCg or EGC. The tendency for a decrease in the catalase activity, which was a result of the paraquat feeding, was suppressed by supplementing EGCg and EGC in the paraquat diet.

Discussion

The protection of EGCg and EGC from decreases in food intake 7 days after the feeding period and in body weight gain 8 days after the feeding period indicate the possibility that EGCg and EGC act preventively against paraquat toxicity which causes oxidative damage to such organs as the lungs, the liver, the kidneys and the heart (Barabás *et al.*, 1984; Matkovics *et al.*, 1980). Stronger protective effects of EGCg foward the decreases in food intake and body weight gain, compared to the protective effect of EGC on those factors, suggest that EGCg may be more effective than EGC in preventing oxidative stress by paraquat.

Although the serum TBARS concentration was not affected to a statistically significant degree by feeding the paraquat diet and by supplementing EGCg and EGC in the paraquat diet, the liver TBARS concentration was increased by feeding the paraquat diet. This indicates that severe loading of the oxidative stress ranging over a period of 10 days due to the paraquat induced lipid peroxidation in the liver. The protective effects of EGCg and EGC against the increase in the liver TBARS concentration by paraquat indicate that both EGCg and EGC act preventively against the oxidative stress induced by paraquat. However, the lung TBARS concentration in the paraquat-fed rats did not differ from that of the control rats, in spite of the fact that the lungs are well-known to be an organ which accumulates the most paraquat. (Bismuth *et al.*, 1990).

Antioxidative enzyme activities in the erythrocytes and liver cytosol fraction were not affected to a statistically significant degree by the paraquat feeding and by supplementing EGCg and EGC in the paraquat diet. One reason for this result might be that the rats fed the paraquat diet in this experiment received stronger oxidative stress, because it is known that antioxidative enzyme activities of the liver and the other organs in the mice received stronger oxidative stress equal to or rather less than those of control rats. (Matkovics *et al.*, 1980). Almost the same antioxidative enzyme activities in the liver of rats fed the paraquat diet and the paraquat diet

supplemented with EGCg or EGC, with the exception of the catalase activity in the mitochondrial fraction, may indicate that EGCg and EGC almost completely prevented the oxidative stress by the paraquat, because it is reported that a moderate or a somewhat severe level of oxidative stress induces increases in the antioxidative enzyme activities (Maktovics *et al.*, 1980).

Marked decreases in the catalase activity in the liver mitochondrial fraction due to the paraquat feeding may suggest that catalase may be an enzyme susceptible to the oxidative stress by paraquat. However, it is necessary to investigate this phenomenon further because that it is known that catalase activity in the mitochondria is generally weak and that mitochondrial fraction prepared by centrifugation in this experiment may be contaminated with peroxisome, in which catalase activity is strong. Mitigation in catalase activity in the mitochondrial fraction by supplementing EGCg and EGC may be a result of the protective effect of these compounds against oxidative stress by the paraquat. Some decrease in the lung catalase activity caused by the paraquat diet and the mitigation in the decrease by supplementing EGCg and EGC in the paraquat diet also suggest that these compounds may be available to prevent oxidative stress by the paraquat.

The decrease in the glutathione and cysteine concentrations in the paraquat-fed rats, compared to the control rats, and the return from decreases in those concentrations by supplementing EGCg or EGC in the paraquat diet suggest that EGCg and EGC prevent the consumption of reduced glutathione and cysteine which may be caused by the paraquat feeding. Stronger suppressive effects of EGCg, compared to EGC, on the decrease in reduced glutathione and cysteine, indicate that the gallic acid moiety of the EGCg molecule is important for the stronger activity of EGCg.

Although the serum and liver lipid concentrations, with the exception of liver triacylglycerol concentration, were not significantly different between the control and paraquat-fed rats, the liver triacylglycerol markedly decreased in the paraquat-fed rats. This indicates that the triacylglycerol concentration may be most strongly affected by the oxidative stress due to paraquat. The decrease in the liver triacylglycerol concentration in the paraquat-fed rats, in comparison to the level of the control rats, may have been due to the inhibition of lipid synthesis by paraquat (Kornbrust & Mavis, 1980) and/or the use of this lipid as an energy source which may be necessary in the rats which cannot retain their body weight. Mitigation in the decrease in the liver triacylglycerol concentration by supplementing EGCg and EGC indicate that these compounds are effective in preventing the decrease in triacylglycerol which was caused by the paraquat. The protective effect of EGCg on the decrease in the triacylglycerol, which is stronger than that of EGC, may further support the belief that the gallic acid moiety in the EGCg molecule plays an important role in demonstrating stronger activity.

References

Barabás, K., Szabó, L., Matkovics, B. and Berencsi, G. (1984). Effects of paraquat on peroxide metabolism enzymes and lipid peroxidation in the rats. Gen. Pharmacol., 15, 133-137.

Bell, G.D. and White, D.A. (1992). The Inhibition of the oxidation of low density lipoprotein by (+)-catechin, a naturally occurring flavonoid. Biochem. Pharmacol., 43, 445-450.

Bismuth, C., Garnier, R., Baud, F.J., Muszynski, J. and Keyes, C. (1990). Paraquat poisoning, an overview of the current status. Drug Safety, 5, 243-251.

Burstein, M., Scholnick, H.R. and Morfin, R. (1970). Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J. Lipid Res., 11, 583-595.

Chen, C.-W. and Ho, C.-T. (1995). Antioxidant properties of polyphenols extracted from green and black teas. J. Food Lipids, 2, 35-46.

Del Boccio, G., Lapenna, D., Porreca, E., Pannelli, A., Savini, F., Feliciani, P., Ricci, G. and Cuccurulle, F. (1990). Aortic antioxidant defence mechanism: time-related changes in cholesterol-fed rabbits. Atherosclerosis, 81, 127-135.

Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226, 497-509.

Frankel, E.N., Kanner, J., German, J.B., Parks, E. and Kinsella, J.E. (1993). Inhibition of oxidation of human low density lipoprotein by phenolic substances in red wine. Lancet, 341, 454-457.

Fukuyo, M., Hara, Y. and Muramatsu, K. (1986). Effects of tea leaf catechin, (−)-epigallocatechin gallate, on plasma cholesterol level in rats. Nippon Eiyo Shokuryo Gakkaishi, 39, 495-500 (in Japanese).

Hara, Y., Matsuzaki, S. and Nakamura, K. (1989). Antitumor activity of tea catechins. Nippon Eiyo Shokuryo Gakkaishi, 42, 39-45 (in Japanese).

Hara, Y. (1994). Prophylactic functions of tea polyphenols. In "Food Phytochemicals for Cancer Prevention II," ed. by C-T. Ho, T. Osawa, M.-T. Huang and R.T. Rosen. American Chemical Society, 547, Washington, DC, pp. 34-50.

He, P. and Yasumoto, K. (1990). Age-associated changes in glutathione peroxidase and oxidized protein in erythrocytes of senescence-accelerated mice. Nippon Eiyo Shokuryo Gakkaishi, 43, 121-125 (in Japanese).

Imanari, T., Hirata, M., Miyazaki, M. and Hayakawa, K. (1977). Improved assay method for superoxide dismutase. Igaku no Ayumi, 101, 496-497 (in Japanese).

Kada, T., Kaneko, K., Matsuzaki, S., Matsuzaki, T. and Hara, Y. (1985). Detection and chemical identification of natural bio-antimutagens, a case of the green tea factor. Nutr. Res., 150, 127-135.

Kornbrust, D.J. and Mavis, R.D. (1980). The effect of paraquat on microsomal lipid peroxidation in vitro and in vivo. Toxicol. Appl. Pharmacol. 53, 323-332.

Lawrence, R.A. and Burk, R.F. (1976). Glutathione peroxidase activity in selenium-deficient rat liver. Biochem. Biophys. Res. Commun., 71, 952-958.

Lowry, O.H., Rosenbrough, N.J., Farr, A.J. and Randahl, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265-275.

Matkovics, B., Szabó, L., Varga, S.I., Barabas, K. and Berencsi, G. (1980). In vivo effects of paraquat on some oxidation in mice and experiments to support the defence against the poisoning. Dev. Biochem., 11B, 367-380.

Paynter, D.I., Moir, R.J. and Underwood, E.J. (1979). Changes in activity of the Cu-Zn superoxide dismutase enzyme in tissues of the rat with changes in dietary copper. J. Nutr., 109, 1570-1576.

Shiraki, S. and Hara, Y. (1990). Antioxidative activity of tea catechins. Fragrance J., 18, 24-30.

Susanne, V., Barbara, T., Davids, A., Gerald, W., Mary, K., Julie, W. and Jose, G. (1996). Inhibitory effect of six green tea catechins and caffeine on the growth of four selected human tumor cell lines. Anti-Cancer Drugs, 7, 461-468.

Suzuki, O., Araki, Y., Igarashi, K., Yoshiki, Y. and Okubo, K. (1997). Protective effects of epigallocatechin gallate on paraquat-induced oxidative stress in rats. Food Sci. Technol. Int. Tokyo, 3, 150-153.

Takahashi, H., Nara, Y., Meguro, H. and Tsujimura, K. (1979). A sensitive fluorometric method for the determination of glutathione and some thiols in blood and mammalian tissues by high performance liquid chromatography. Agric. Biol. Chem., 43, 1439-1445.

Teissedre, P.L., Frankel, E.N., Waterhouse, A.L., Pelag, H. and German, J.B. (1996). Inhibition of in vitro LDL oxidation by phenolic antioxidants from grapes and wines. J. Sci. Food Agric., 70, 55-61.

Toda, H., Okubo, S., Hara, Y. and Shimamura, T. (1991). Antibacterial and bactericidal activities of tea extracts and catechins against methicillin resistant Staphylococcus aureus. Nippon Saikingaku Zasshi, 46, 839-845 (in Japanese).

Tomita, K. and Sano, M. (1983). In "Kasanka Shishitsu Jikkenho," ed. by T. Kaneda, and N. Ueda. Ishiyaku Shuppan Co., Tokyo, pp. 149-150 (in Japanese).

Uchiyama, M. and Mihara, M. (1978). Determination of malonaldehyde precursor in tissue by thiobarbituric acid test. Anal. Biochem., 86, 271-278.

Yagi, K. (1976). A simple fluorometric assay for lipoperoxide in blood plasma. Biochem. Med., 15, 212-216.

Yen, G.-C. and Chen, H.-Y. (1995). Antioxidative activity of various tea extracts in relation to their antimutagenecity. J. Agric. Food Chem., 43, 27-32

Yoshino, K., Tomita, I., Sano, M., Oguni, I., Hara, Y. and Nakano, M. (1994). Effects of long-term dietary supplement of tea polyphenols on lipid peroxide levels in rats. Age, 17, 79-85.

Yoshizawa, S., Horiuchi, T. and Fujiki, H. (1987). Antitumor promoting activity of (−)-epigallocatechin gallate, the main constituent of "Tannin" in green tea. Phytother. Res., 1, 44-47.