Hypolipidemic Effect of Green Tea Leaves through Induction of Antioxidant and Phase II Enzymes Including Superoxide Dismutase, Catalase, and Glutathione *S*-Transferase in Rats

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In this animal study, Wistar rats were fed 2.5% green tea (longjing) leaves, for 27 and 63 weeks; the changes of GOT, GPT, γ-GT, and creatinine were not significant in the treated group as compared with the control. These results suggested that long-term feeding of green tea leaves was not toxic to the liver or kidney. Serum total cholesterol, triglyceride, and LDL-C were decreased in the tested group. Interestingly, the dietary intakes of the two groups were approximately the same, but the body weights of the tea-fed group were decreased 10−18% compared with those of the control. The activities of antioxidant enzymes (SOD and catalase) and phase II enzyme (GST) and glutathione concentration in the liver of Wistar rats were significantly higher in the treated group. The biological significance of these results can be implicated in relation to the hypolipidemic effect as well as the cancer chemopreventive action of green tea.

Keywords: *Longjing tea; GST; HDL-C; hypolipidemic; LDL-C; phase II enzyme; SOD*

INTRODUCTION

Tea plants are widely cultivated in Southeast Asia. Tea is one of the most popular beverages in the world because of its attractive flavor, aroma, and taste. Over 300 different kinds of tea are now produced, but there are only 3 general forms of tea: the unfermented green tea, the partially fermented paochong tea or oolong tea, and the fermented black tea. Green tea is manufactured by steaming or drying fresh tea leaves to prevent oxidation of the green tea polyphenols (Takeo, 1992). The manufacture of black tea is characterized by a high degree of enzymatically catalyzed aerobic oxidation of the leaf polyphenols followed by a series of chemical condensations (Hampton, 1992). Paochong tea or oolong tea is partially oxidized (Takeo, 1992). The composition of tea varies with species, season, the age of the leaf (plucking position), climate, and horticultural practices. Green tea contains polyphenols, which include flavanols, flavandiols, flavonoids (Hertog et al., 1993), and phenolic acids; these compounds may account for up to 30% of the dry weight. The polyphenols are the most significant group of tea components, especially certain catechins. The major tea catechins are (−)-epigallocatechin 3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin 3-gallate (ECG), (−)-epicatechin (EC), (+)-gallocatechin, and (+)-catechin (C).

Many biological functions of tea polyphenols have been studied (Yang and Wang, 1993), including antioxidative activity (Ho et al., 1992; Katiyar et al., 1994; Yen et al., 1995; Lin et al., 1996), anti-inflammation (Lin and Lin, 1997), antimutagenic (Wang et al., 1989; Shiraki et al., 1994), and anticarcinogenic effects (Oguni et al., 1988; Wang et al., 1992; Katiyar et al., 1993), lowering of plasma cholesterol and triglyceride levels, and reduction of blood pressure and platelet aggregation (Muramatsu et al., 1986; Chisaka et al., 1988) in several systems.

Yang et al. (1993) reported that a cup (200 mL) of green tea (Gunpowder, Hangzhou, China) contains about 142 mg of EGCG, 65 mg of EGC, 28 mg of ECG, 17 mg of EC, and 76 mg of caffeine. In 1996, our laboratory (Lin et al., 1996) analyzed 10 different types of commercial tea (manufactured tea), including unfermented, semifermented, and fermented tea for their polyphenol compounds, and it was found that the amount of EGCG in tea water extracts varied with different tea leaves and processing methods. We found that Longjing tea (unfermented green tea) contained the highest concentration of EGCG and polyphenols. A cup (200 mL) of 1.25% longjing tea contains about 305 mg of EGCG, 145 mg of EGC, 70 mg of ECG, 28 mg of EC, 8 mg of C, 1 mg of GA, and 142 mg of caffeine. We also found that EGCG more strongly inhibited the peroxyl radical generation than other tea polyphenols. Therefore, in this study we selected longjing tea for the rat-feeding experiment.

In the present study, we have investigated the effect of green tea leaves on the levels of cholesterol, antioxidant, and phase II enzymes in Wistar rats by oral feeding. The data suggest that oral feeding of green tea leaves to rats can result in the reduction of total cholesterol, triglyceride, and LDL-C and the enhancement of activities of SOD in serum and phase II enzymes GST and catalase in the rat liver.

MATERIALS AND METHODS

Chemicals. NADPH, H_2O_2, 1-chloro-2,4-dinitrobenzene, and oxidized and reduced glutathione were purchased from Sigma Chemical Co. (St. Louis, MO). Reagent kits for GOT,

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GPT, γ-GT, creatinine, total cholesterol, triglyceride, LDL-C, and HDL-C were purchased from E. Merck Co. (Darmstadt, Germany). All other chemicals and reagents used were of the highest purity commercially available. Chinese green tea leaves, longjing tea, were purchased from Wangs' Tea Enterprise Co. Ltd. (Taipei, Taiwan) and stored at 4 °C in a sealed bag. In the experiment, longjing tea was crushed into powder and thoroughly mixed with the basal diet. The data and analytical methods for the composition of polyphenols in longjing tea leaves have been published in 1996 by our laboratories (Lin et al., 1996).

Animals and Treatments. Male Wistar rats (5 weeks old) were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). The rats were housed in stainless steel wire-bottomed cages and acclimatized under laboratory conditions (19−23 °C, humidity 60%, 12 h light/dark cycle) for at least 1 week before each study. At the end of this period, rats were weighed and randomly assigned to one of two groups. The weights of rats at the beginning of the study ranged from 120 to 160 g. All rats were weighed twice during each month of the study. Free access to ground Purina rat chow (Ralston Purina Co., Inc., St. Louis, MO) and water was permitted prior to the experimental period. After 1 week of acclimatization, the rats were fed different diets: group 1, 2.5% green tea leaves (longjing tea); group 2, basal diet (ground Purina rat chow). The experiment was terminated after 63 weeks. The rats were then ether-anesthetized, blood was collected from the jugular vein, serum was separated for the estimation of GOT, GPT, γ-GT, creatinine, total cholesterol, triglyceride, LDL-C, and HDL-C, and the liver and kidney were quickly excised and frozen at −70 °C until use.

Enzyme Assay. Serum GOT and GPT activities were determined according to the method of Reitman and Frankel (1957). Serum was added to a buffer solution of α-ketoglutaric acid and aspartic acid or alanine, and the resulting oxaloacetic aicd or pyruvic acid formed after incubation was measured colorimetrically at 546 nm by reaction with dinitrophenylhydrazine. The activity of γ-GT in serum was measured according to the method of Persijin and van der Slik (1976). Serum was incubated with L-γ-glutamyl-3-carboxy-4-nitroanilide and glycylglycine as substrates, and the resulting 5-amino-2-nitrobenzoate was determined spectrophotometrically at 405 nm. Enzyme units were expressed as micromoles of substrate metabolized per minute and defined as units per liter.

Creatinine Assay. The content of creatinine was determined in serum according to the Jaffe method. Creatinine forms a yellow-orange compound in alkaline solution with picric acid. At the low picric acid concentration used in this method a precipitation of protein does not take place. The quantity of the prepared quinonimine dye formed is proportional to the creatinine concentration and is measured photometrically at 510 nm (Siedel et al., 1984).

Triglycerides Assay. This was done by the GPO−PAP method. Triglycerides are enzymatically hydrolyzed to glycerol and free fatty acids by special lipases. In the subsequent enzymatic oxidation by glycerol kinase and glycerol phosphatase, H_2O_2 is formed. This is converted into a colored quinonimine in a reaction with 4-aminoantipyrine and phenol catalyzed by peroxidase, which was determined spectrophotometrically at 546 nm. The unit of the content of triglyceride was expressed as milligrams per deciliter.

Cholesterol Assay. This was estimated by the CHOD−PAP method. Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyzes the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H_2O_2 is formed. This is converted into a colored quinonimine in a reaction with 4-aminoantipyrine and phenol catalyzed by peroxidase, which was determined spectrophotometrically at 546 nm. The unit of the content of cholesterol was expressed as milligrams per deciliter.

HDL-Cholesterol Assay. Low-density lipoproteins (LDL and VLDL) are specifically precipitated by phosphotungstic acid and magnesium ions and can then be removed by centrifugation. HDL remain in the supernatant. Determination of HDL-C is performed using the clear supernatant. This is estimated by the CHOD−PAP method. The unit of the content of HDL-C was expressed as milligrams per decilter.

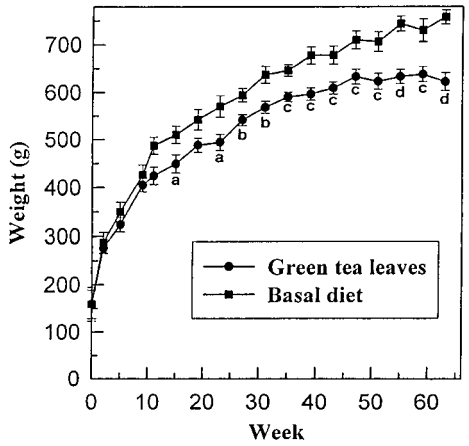

Figure 1. Change of body weights of Wistar rats in the period fed on 2.5% green tea leaves for 63 weeks. The data are presented as mean ± SE from 12 rats per group. (a) Statistically different from the control group ($p < 0.05$); (b) statistically different from the control group ($p < 0.01$); (c) statistically different from the control group ($p < 0.005$); (d) statistically different from the control group ($p < 0.0005$).

LDL-Cholesterol Assay. LDL are precipitated by heparin at their isoelectric point (pH 5.12). After centrifugation, the HDL and the VLDL remain in the supernatant and can then be determined by enzymatic methods. LDL-C = total cholesterol − cholesterol in the supernatant. The unit of the content of LDL-C was expressed as milligrams per deciliter.

Assay of Superoxide Dismutase Activity in Serum. Serum SOD activity was determined according to the method of Nebot et al. (1993). Four hunder microliters of ice-cold absolute ethanol/chloroform 62.5:37.5 (v/v) was added to 250 μL of serum in a glass test tube and then thoroughly mixed for at least 30 s and centrifuged at 3000*g* for 5 min at 4 °C. The resulting supernatant should be stored between 2 and 8 °C until used for the assay. Forty microliters of the supernatant with 900 μL of 0.11 mM diethylenetriaminepentaacetic acid (pH 8.8) and 30 μL of mercaptan scavenger [in DMSO containing 25% ethylene glycol (w/v)] was thoroughly mixed, and the reaction mixture was incubated for 1 min at 37 °C. Thrity microliters of chromogenic reagent (in 32 mM HCl was added and mixed rapidly, and the absorbance at 525 nm against air (reference cuvette) was recorded for 1 min.

Estimation of Antioxidant and Phase II Enzyme Activities and Glutathione. Liver was homogenized in PBS, and a 100000*g* supernatant fraction was prepared as described earlier (Agarwal et al., 1992). SOD activity was determined as described by McCord and Fridovich (1969). Catalase activity was determined by following the decomposition of H_2O_2 measured as a decrease in absorbance at 240 nm (Juch et al., 1989). GST activity was determined according to the method of Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene as a substrate. The content of reduced GSH was quantitated by using the method of Reiners et al. (1991a,b).

Statistical Analysis. The results obtained were expressed as mean ± SE, and the significance of the differences (*p* values) was assessed by Student's *t* test.

RESULTS

Effects of Green Tea Leaves on the Body Weight and Dietary Intake of Rats. The body weights of rats in each group are given in Figure 1. At the 15th week, the average body weights of the green tea leaves-fed group and the basal diet-fed group were 449 and 510 g, respectively. The oral feeding of green tea leaves resulted in a significant 12% decrease in the average

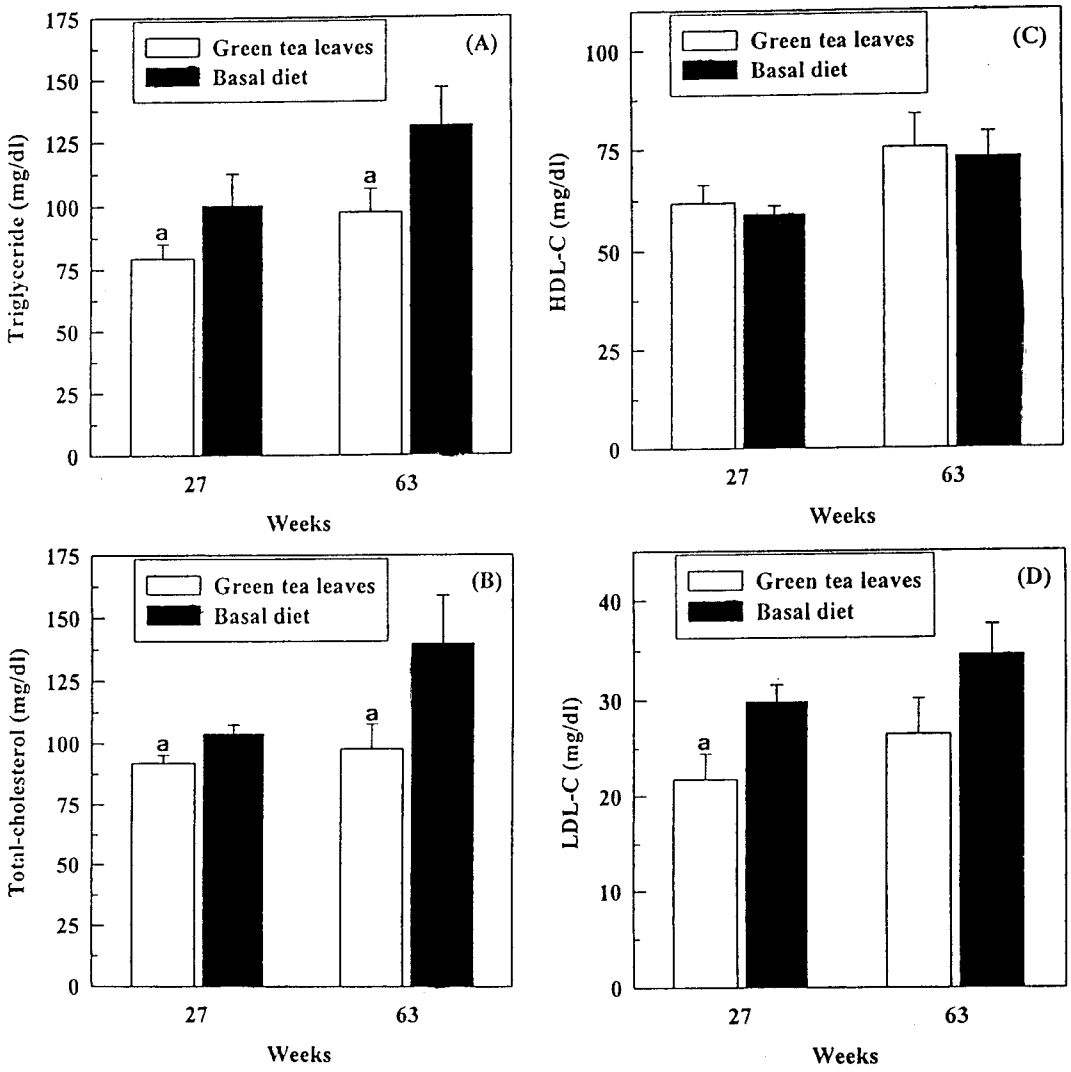

Figure 2. Levels of triglyceride, total cholesterol, HDL-C, and LDL-C in the serum of Wistar rats fed 2.5% green tea leaves for 63 weeks. The data are presented as mean ± SE from 12 rats per group. (a) Statistically different from the corresponding control group ($p < 0.05$).

Table 1. Dietary Intake (Grams per Day per Rat) of Wistar Rats Fed on 2.5% Green Tea Leaves

	week after initiation of treatment								
diet group	1	2	3	4	48	50	52	54	mean ± SE
green tea leaves[a]	23.7	25.0	26.2	26.8	32.5	32.0	31.0	31.3	28.6 ± 1.2
basal diet[a]	23.5	25.9	25.1	26.4	31.9	32.7	31.2	31.6	28.5 ± 1.3

[a] The data are from 12 rats per group.

body weights as compared to that observed in control group, and the levels were statistically significant ($p < 0.05$). Between the 15th and the 63rd week, the average body weights of tea-fed group were 10−18% lower than those of the basal diet-fed group. Table 1 shows the dietary intake of Wistar rats fed on 2.5% green tea leaves. The dose of green tea leaves used in the present study did not reduce diet and water consumption throughout the feeding regimen. The survival ratios of both tea-fed and basal diet-fed groups are 100% (12/12) during the courses of experiments.

Effects of Green Tea Leaves on the Activities of GOT, GPT, and γ-GT. Green tea leaves (2.5%) were added into the diet and given to the experimental animals for 63 weeks. The liver functions of these experimental animals were assayed on the basis of their serum marker enzyme levels (data not shown). The results indicated that rats fed on a diet containing 2.5% green tea leaves for 27 or 63 weeks developed no hepatic functional disorders as demonstrated by the absence of change of serum GOT, GPT, and γ-GT. It seemed that green tea leaves at the concentration of 2.5% did not have any toxic effect on the hepatic tissue as revealed by the normal levels of serum marker enzymes. In histological examination, we did not observe any microscopic lesions in the liver (data not shown).

Effect of Green Tea Leaves on the Serum Creatinine. Serum creatinine is the most useful indicators of kidney injury and diseases. In the case of renal damage the content of creatinine is increased in serum. At the 27th or 63rd week, the concentration of creatinine in rat serum was not significantly different between the tea-fed (2.5% green tea leaves) and basal diet-fed groups (data not shown). In histological examination, we did not observe any microscopic lesions in the kidney (data not shown). Therefore, 2.5% green tea leaves did not show any toxic effect in the kidney.

Effect of Green Tea Leaves on the Serum Triglyceride, Total Cholesterol, HDL-C, and LDL-C. Figure 2 illustrates the levels of triglyceride, total cholesterol, HDL-C, and LDL-C in the serum of Wistar

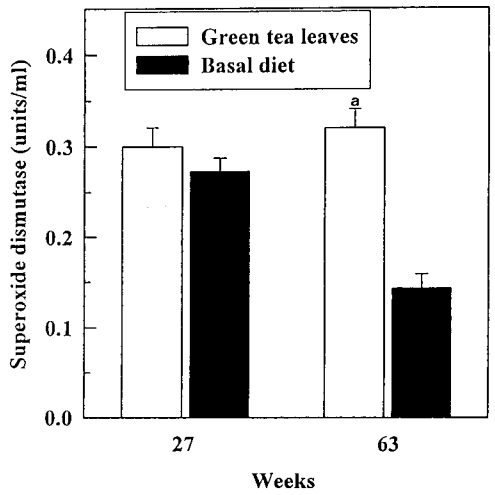

Figure 3. Activity of superoxide dismutase in the serum of Wistar rats fed 2.5% green tea leaves for 63 weeks. The data are presented as mean ± SE from 12 rats per group. (a) Statistically different from the corresponding control group ($p < 0.0005$).

Table 2. Effect of Oral Feeding of 2.5% Green Tea Leaves in Diet to Male Wistar Rat on Antioxidant, Phase II, Enzyme Activities and Glutathione Content in Liver[a] for 63 Weeks

	green tea leaves	basal diet
SOD (units mg^{-1})	54.2 ± 5.7	47.2 ± 2.8
catalase activity [nmol of H_2O_2 consumed (mg of protein)$^{-1}$ min^{-1}]	348.2 ± 34.5[b]	262.5 ± 14.6
GST activity [nmol of 1-chloro-2,4-dinitro-benzene conjugate formed (mg of protein)$^{-1}$ min^{-1}]	2240.4 ± 190.8[b]	1715.9 ± 115.7
GSH [nmol (mg of protein)$^{-1}$]	268.7 ± 12.6	224.8 ± 24.2

[a] Data represent mean ± SE ($n = 12$). For statistical significance, Student's t test was used between normal diet-fed control and green tea leaves-fed experimental group. [b] Statistically significant versus control; $p < 0.05$.

rats fed 2.5% green tea leaves for 63 weeks. The results demonstrated that the contents of triglyceride and total cholesterol in the tea-fed group were significantly lower than those of the basal diet-fed group at the 27th and 63rd weeks ($p < 0.05$) (Figure 2A,B). Figure 2C shows the concentration of HDL-C in the serum of the tea-fed group was slightly increased as compared to that of the basal diet-fed group, but the differences were not statistically significant ($p > 0.05$). At the 27th week, we also found that the content of LDL-C in the tea-fed group was markedly decreased as compared to the basal diet-fed group ($p < 0.05$) (Figure 2D).

Effect of Green Tea Leaves on the Activity of Superoxide Dismutase in Serum. Figure 3 shows that green tea leaves could enhance the activity of SOD in serum as compared to the basal diet-fed group ($p < 0.0005$) at the 63rd week.

Effects of Green Tea Leaves on the Activities of Catalase and Phase II Enzyme in Liver. The data in Table 2 show the activities of SOD, catalase, and GST and glutathione content in liver of both the tea-fed and the normal basal diet-fed rats. The oral feeding of green tea leaves resulted in a significant increase in the activities of catalase and GST in the liver as compared to the control group. The activity of SOD and glutathione content in liver were also found to be elevated, but the levels were not statistically significant when compared with those obtained in the control group.

DISCUSSION

The association of plasma cholesterol and lipoprotein concentrations with atherosclerosis has been investigated by several workers. Increased levels of plasma cholesterol, LDL-C, and VLDL-C are risk factors contributing to the development of coronary heart diseases (Hollman et al., 1996) and artherosclerosis (Reaven and Witztum, 1996). In this study, lowered serum total cholesterol, triglyceride, and LDL-C were observed when 2.5% green tea leaves were supplemented to the basal diet. Our results strongly suggested that green tea leaves exerted a hypolipidemic effect and therefore might have a protective effect against the atherosclerotic process. It has also been reported that tea catechins have hypocholesterolemic effects in experimental animals (Muramatsu et al., 1986). Muramatsu et al. (1986) observed that dietary green tea catechins increased fecal excretions of cholesterol and total lipids in cholesterol-fed rats. Chisaka et al. (1988) showed that orally administered EGCG decreased cholesterol absorption from rat intestine. Ikeda et al. (1992) also found that tea catechins, in particular their gallate esters (EGCG or ECG), effectively reduced cholesterol absorption from the intestine by reducing the solubility of cholesterol in mixed micelles. Therefore, in Figure 2B demonstrates that the content of total cholesterol in the tea-fed group was significantly lower than that of the basal diet-fed group. In Table 1 and Figure 1, we found that the dietary intakes of the two groups were approximately the same, but the body weights of the tea-fed group were 10−18% lower than those of control group. Therefore, we proposed two reasons for the lowered body weight in the tea-fed group: one reason may the enhanced excretion of total lipids by tea catechins; the other possible reason may be attributed to an increased metabolic rate caused by caffeine (Spurlock et al., 1996; Caraco et al., 1995). The catechins are the most abundant group of compounds in green leaves. Moreover, other components in green tea leaves, such as absorbic acid, tocopherol, β-carotene, chlorophyll, and fiber, were also found to be very important. The amounts of these components in tea may vary with the variety, harvesting season, and processing method, which may affect the antimutagenic, anticarcinogenic, and antihyperlipidemic activities (Sun et al., 1997; Poppel and Berg, 1997; Jacob and Burri, 1996; Lairon, 1996; Trusweel, 1995).

Reactive oxygen species might be important causative agents for a number of human diseases, cancer, atherosclerosis, and aging. Tea polyphenols such as EGCG, EGC, and ECG could react with peroxyl radical and thus terminate lipid peroxidation chain reactions (Katiyar et al., 1994). We have found that EGCG has more a potent scavenged peroxyl radical action than other tea polyphenols (Lin et al., 1996). In this study, we found that the SOD activity in the serum was increased in the tea-treated group. Then the SOD can remove the superoxide anion radicals (this radical can produce cytotoxicity and genotoxicity). Recently, Nakagawa and Miyazawa (1997) established the chemiluminescence detection−high-performance liquid chromatography (CL-HPLC) method to measure plasma EGCG with high selectivity and sensitivity (at picomole level). The data demonstrated the absorption of EGCG in the free form

into rat and human plasma. Therefore, EGCG can direct function in our circulation system.

LDL oxidation plays an important part in the development of atherosclerosis (Steinberg, 1997). Oxidized LDL could promote atherogenesis by its cytotoxicity, its chemotactic effects on monocytes, its inhibitory effects on macrophage motility, and its uptake by the macrophage scavenger receptor, resulting in the stimulation of cholesterol accumulation and hence foam cell formation. There are several studies suggesting that naturally occurring antioxidants in the diet may play a role as antiatherosclerotic agents. Mangiapane et al. (1992) reported that (+)-catechin inhibited the Cu^{2+}-catalyzed oxidation of human LDL in a dose-dependent manner with complete inhibition at 20 μg/ mL. (+)-Catechin also inhibited the oxidation of LDL induced by the mouse transformed macrophage J774, human monocyte-derived macrophages, and vascular endothelial cells isolated from human umbilical cords. Similarly, Miura et al. (1994) also found that EGCG suppressed the Cu^{2+}-catalyzed oxidative modification of LDL. Recently, Luo et al. (1997) reported the inhibition of LDL oxidation by green tea extract. The mechanisms by which tea polyphenols inhibit Cu^{2+}-mediated LDL oxidation are unclear at present. They might reduce the formation of free radicals, or they might protect α-tocopherol and other antioxidants in LDL, maintaining their levels longer and delaying the start of lipid peroxidation (Esterbauer et al., 1987, 1989; Bedwell et al., 1989).

Nitric oxide and superoxide, produced by a variety of cells including activated neutrophils and macrophages, react to form peroxynitrite ($ONOO^-$) (Rodenas et al., 1995). The nitration of tyrosine to 3-nitrotyrosine and oxidation of LDL by peroxynitrite have been suggested as initiating steps in atherosclerosis (Graham et al., 1993). In 1997, Pannala et al. reported that tea polyphenols could decrease the nitrosation of tyrosine and limit modification of LDL induced by peroxynitrite. They found that EGCG, ECG, and gallic acid are the most effective peroxynitrite scavengers. In 1997, our laboratory reported that EGCG can block the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor NF-κB in macrophages (Lin and Lin, 1997). In the present study, we found that the SOD activity in the serum was increased in the tea-treated group. The SOD can remove the superoxide anion radicals. Therefore, EGCG can reduce the nitric oxide and superoxide anion radical generation and then decrease peroxynitrite ($ONOO^-$) formation (Lin and Lin, 1997; Yen and Chen, 1995).

Many previous studies have focused on the anticarcinogenic and antimutagenic effects of tea. Furthermore, the mechanisms contributing to the anticarcinogenic and antimutagenic effects of tea may involve the antioxidative activity (Ho et al., 1992; Katiyar et al., 1994; Yen et al., 1995; Lin et al., 1996), induction of phase II enzymes (Khan et al., 1992), blocking of the biosynthesis of ultimate carcinogen (Nakamura and Kawabata, 1981), inhibition in the covalent binding of carcinogen to DNA (Shi et al., 1994), and inhibition of DNA synthesis and cell proliferation (Lea et al., 1993). In addition, the levels of antioxidant defense enzymes such as SOD and catalase are also known to be lower in transformed cells and/or tumors (Sun et al., 1990; Perchellet and Perchellet, 1989). Reiners et al. (1991a,b) have shown the depleted levels of antioxidant enzymes in 7,12-dimethylbenz[*a*]anthracene-12-*O*-tetradecanoylphorbol-13-acetate-treated skin and in skin tumors induced chemically. In the present study, we found that oral feeding of green tea leaves to rats results in the enhancement of activities of SOD in serum and of phase II enzymes GST and catalase in liver. Similar observations were made by Khan et al. (1992) when SKH-1 hairless mice were treated for 30 days with polyphenols (0.2%, w/v) isolated from green tea in drinking water; increases were observed in the hepatic, pulmonary, and small bowel catalase and glutathione peroxidase activities. Bu-Abbas et al. (1995) have shown that treatment of rats with green tea aqueous extracts (2.5%, w/v) as the sole drinking fluid for 4 weeks, at concentrations consumed by humans, can increase UDP-glucuronosyl transferase activity. In 1995, our laboratory also found that after the intraperitoneal injections of green tea polyphenols (2.5%, w/v) to rats the activities of antioxidant and phase II enzymes were elevated (Lee et al., 1995).

Taken together, our results show that oral feeding of green tea leaves to rats results in the reduction of triglyceride, total cholesterol, and LDL-C and the enhancement of activities of SOD in serum and phase II enzymes GST and catalase in liver. The significance of these results can be implicated in relation to the hypolipidemic effect and the cancer chemopreventive action of green tea against the induction of tumors.

ABBREVIATIONS USED

C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin 3-gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin 3-gallate; GA, gallic acid; γ-GT, γ-glutamyltransferase; GSH, glutathione; GST, glutathione *S*-transferase; HDL-C, high-density lipoprotein−cholesterol; LDL-C, low-density lipoprotein-cholesterol; SOD, superoxide dismutase.

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