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Inhibitory effect of jasmine green tea epicatechin isomers on LDL-oxidation

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Oxidative modification of low-density lipoproteins (LDL) is thought to play an important role in the development of atherosclerosis. The present study examined inhibitory effect of jasmine green tea polyphenol (GTP) extracts and its individual epicatechin isomers on Cu^{+2}-mediated oxidation of human LDL. The jasmine tea GTP extracts consisted mainly of four epicatechin isomers with (−) epigallocatechin gallate (EGCG) being most abundant (51.2%) followed by (−) epigallocatechin (EGC, 18.7%), (−) epicatechin (EC, 12.3%), and (−) epicatechin gallate (ECG, 11.8%). Jasmine tea GTP as a mixture or its four epicatechin isomers demonstrated strong antioxidant activity in Cu^{+2}-mediated oxidation of human LDL. The inhibitory effect of these epicatechin isomers on LDL oxidation was dose-dependent at the concentrations ranging from 5 to 40 μM. EC and EGC were less protective against LDL oxidation than their gallate derivatives, ECG and EGCG, respectively. ECG and EGCG seemed to have a similar antioxidant activity. In contrast, EGC was less effective than EC as an antioxidant against LDL oxidation. The inhibitory effect of jasmine green tea epicatechin isomers on Cu^{+2}-mediated oxidation was also characterized by protecting docosahexaenoic, arachidonic, α-linolenic, and linoleic acid in LDL against oxidative degradation. We conclude that, in addition to their hypocholesterolemic effect, jasmine tea epicatechin isomers may serve as a source of natural antioxidative agents in human diet. (J. Nutr. Biochem. 8: 334–340, 1997) © *Elsevier Science Inc. 1997*

Keywords: epicatechin; epicatechin gallate; epigallocatechin; epigallocatechin gallate; jasmine tea

Introduction

It has been suggested that oxidative modification of low-density lipoprotein (LDL) may play a role in the development of atherosclerosis.[1–4] This hypothesis is supported by the observations that oxidatively modified LDL is present in atherosclerosis plague but absent in normal artery wall.[5–7] There is increasing interest in green tea polyphenols (GTP) as dietary antioxidants against oxidation of LDL in vivo.[8,9] In addition, increased consumption of green tea has been shown to be negatively associated with serum total cholesterol and triacylglycerols.[10–12] Effect of drinking green tea on plasma lipoproteins is characterized by decreasing LDL cholesterol while increasing high-density lipoprotein cholesterol.[11,12] In contrast, drinking black tea seems to have no effect on plasma total cholesterol.[13,14] This suggests that the beneficial effect of drinking green tea over black tea is attributed to the content of GTP because in the former, GTP remain unchanged, whereas they are degraded by the fermentation process in the latter.

We have examined previously the antioxidative properties of the tea ethanol extracts and found that the ethanol extracts of green tea and white tea exhibited a stronger inhibition on lipid oxidation in canola oil than butylated hydroxytoluene.[15] In contrast, the ethanol extracts from black tea and dark-green tea showed no or little antioxidative activity.[15] The varying protection of tea ethanol extracts against lipid oxidation may be attributable to the distinct manufacturing processes. Green tea and white tea are nonfermented products in which GTP are mostly preserved, whereas GTP in black tea and dark-green tea are oxidized extensively to form browning polymers either by polyphenol oxidase or nonenzymatic browning reactions.[16] Several major epicatechin isomers can be found in green tea extracts including mainly (−) epicatechin (EC), (−) epicatechin gallate (ECG), (−) epigallocatechin (EGC), and (−) epigallocatechin gallate (EGCG). We have studied previously

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their relative antioxidative activity and found that EGC is most effective as an antioxidant followed by EGCG, EC, and ECG in cooking oil.[17]

Jasmine tea is one of the most popular beverages consumed in China. However, information on its GTP composition and possible health benefits is limited. The objectives of the present study were: (1) to quantify the GTP composition of jasmine tea; (2) to isolate and purify each individual GTP isomers from jasmine tea; and (3) to examine the antioxidative activity of jasmine tea GTP either as a mixture or separately on human LDL oxidation in vitro.

Methods and materials

GTP extraction

The method described by Agarwal et al.[18] was modified and used to extract total GTP from jasmine tea. In brief, 10 g of jasmine tea leaves were soaked three times with 140 mL of hot distilled water (80°C). The infusion was cooled to room temperature, filtered, and then extracted with equal volume of chloroform to remove caffeine and pigments. After removing the chloroform phase, the remaining aqueous layer was extracted twice with equal volume of ethyl acetate. The ethyl acetate phase containing GTP was then saved and ethyl acetate was removed using a vacuum rotary evaporator. The resulting crude GTP extracts were dissolved in 10 mL of distilled water and freeze-dried overnight.

HPLC analysis of GTCs

The individual GTP isomers in jasmine tea extracts were separated using a Shimazu LC-10AD HPLC (Tokyo, Japan) equipped with a ternary pump delivery system. In brief, 15 μL of jasmine tea extracts (2 mg/mL) was injected onto the column (Microsorb MV, 250 × 4.6 mm, 5 μm, Rainin, Woburn, MA, USA) via a rheodyne valve (20 μL capacity, Shimadzu, Tokyo, Japan). A gradient of methanol in water was used at a flow rate of 0.7 mL/min (0 to 7 min, 28% methanol changing to 40%; 7 to 14 min, 40% methanol changing to 52%; 14 to 20 min, 52% methanol changing to 28%). The separated GTP isomers were monitored using an evaporative light scattering detector (Model MK III, Alltech, Burtonsville, MD, USA) and quantified using (+) catechin as an internal standard. Individual GTP isomers were identified by comparing the retention time of known standards or adding known standards to the sample. A typical HPLC chromatogram of jasmine tea GTP extracts is shown in *Figure 1*.

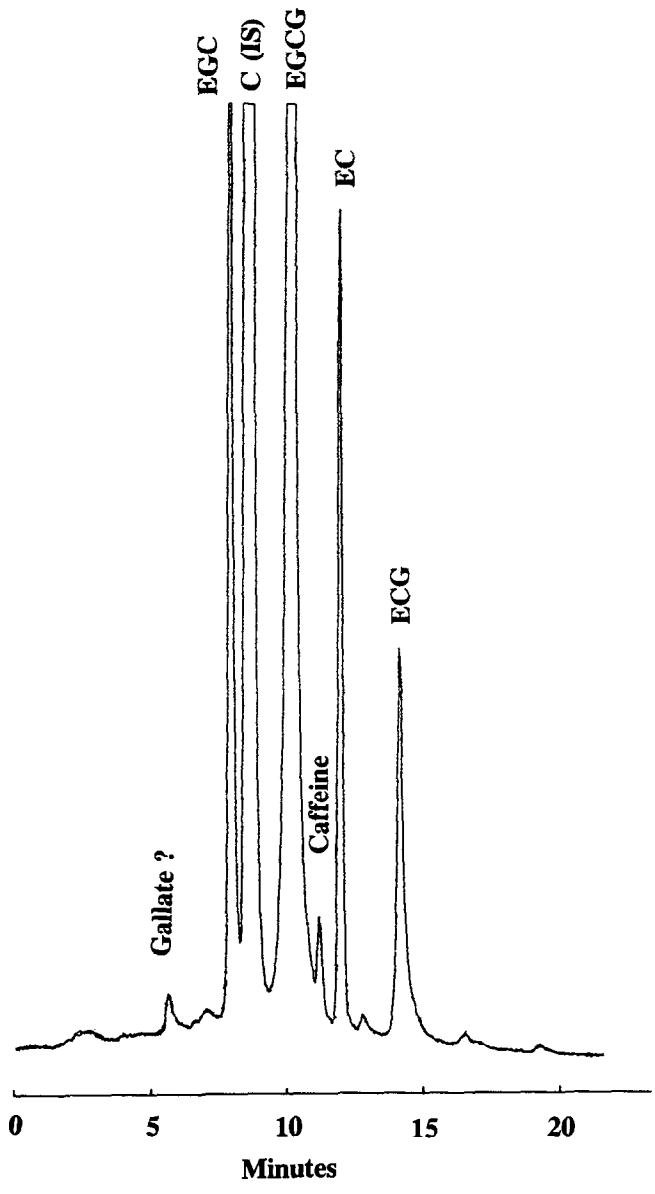

Figure 1 HPLC profile of jasmine green tea polyphenols. See text for the conditions and *Table 1* for percent composition. Peak identifications: EGC, (−) epigallocatechin; C, (+) catechin (internal standard); EGCG, (−) epigallocatechin gallate; EC, (−) epicatechin; and ECG, (−) epicatechin gallate.

Isolation and purification of individual GTP isomers

Individual jasmine tea GTP isomers were isolated using a semi-preparative column (Spherisorb ODS-1, 250 × 10 mm, 10 μm, Isco, Inc., Lincoln, NE, USA). In brief, 50 mg GTP in H_2O was loaded onto the column via a rheodyne valve with a 250 μL sample loop. A 29% methanol solution in H_2O was used at a flow rate of 0.7 mL/min. The eluting peaks were monitored at 280 nm using a UV detector (UVIS-205, Alltech, Deerfield, IL, USA) and collected manually. The fraction containing GTP isomers was checked immediately using an analytic column as described previously and the purity of each isomer isolated was found to be >99%. The methanol was then removed using a rotary evaporator. The resulting pure GTP isomers were then freeze-dried overnight and stored in dark at −20°C until used.

LDL isolation

Fresh blood was collected from healthy subjects at the Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong. To prevent lipoprotein modification, EDTA and NaN_3 solutions were added to freshly prepared plasma (final concentrations of EDTA and NaN_3 were 0.1% and 0.05%, respectively). LDL was isolated from plasma according to the method described previously.[19] To minimize the oxidation of LDL, the centrifuge tube containing plasma was flushed with nitrogen. Briefly, the plasma was firstly centrifuged at 1,500 g for 15 min to remove cells and cell debris. Plasma density was then increased to 1.019 by addition of a NaCl-KBr solution (dissolve 153 g NaCl, 354 g KBr and 100 μg EDTA in one liter of H_2O, 1.33g/mL) and recentrifuged at 160,000 g at 10°C for 20 hr. After removing the top layer containing chylomicron and very low-density lipoprotein, the density of remaining plasma fractions was increased to 1.064 and recentrifuged at 160,000 g for an additional 24 hr. The top LDL fraction was collected and then flushed with nitrogen and

stored at −70°C. The protein content of isolated LDL was determined using Lowry's method.[20]

LDL-oxidation

Before use, the stock LDL fraction (5 mg protein/mL) was dialyzed against 100 volume of the degassed dialysis solution, pH 7.4 containing 0.01 M sodium phosphate, 0.9% NaCl, 10 μM EDTA, and 0.05% NaN_3 in dark for 24 hr. The dialysis solution was changed four times. Oxidation of LDL was conducted as described previously by Puhl et al.[21] In brief, 100 μg LDL protein was incubated in a mixture containing 5 μM $CuSO_4$ and 5 to 40 μM GTP extracts or individual epicatechin isomers at 30°C for up to 36 hr. The oxidation was then stopped by addition of 25 μL 1.0% EDTA and cooled at 4°C.

Thiobarbituric acid-reactive substances (TBARS) assay

The degree of LDL-oxidation was monitored by measuring the production of TBARS as previously described by Buege et al.[22] The LDL-incubated tube was added immediately to 2 mL of 0.67% thiobarbituric acid and 15% trichloroacetic acid in 0.1 N HCL solution. The incubation mixture was then heated at 95°C for 1 hr, cooled on ice, and centrifuged at 1,000 g for 20 min. TBARS were then determined by measuring the absorbance at 532 nm. The calibration was done with a malondialdehyde (MDA) standard solution prepared from tetramethoxylpropane. The value of TABRS was expressed as nmol MDA/mg LDL protein.

Lipid analysis

An additional set of LDL was incubated in a mixture containing 5 μM $CuSO_4$ and 5 μM GTP extracts or individual epicatechin isomers at 30°C for 4 to 12 hr. Total LDL lipids were thereafter extracted using chloroform/methanol (2:1, v/v) containing 0.02% butylated hydroxytoluene (Sigma Chemical, St. Louis, MO USA) as an antioxidant and containing heptadecanoic acid as an internal standard to quantify individual fatty acids. The lipid extracts were then converted to fatty acid methyl esters by using a mixture of 14% BF_3-MeOH (Sigma) and toluene (2:1, vol/vol) under nitrogen at 90°C for 45 min.

The fatty acid methyl esters were analyzed by gas liquid chromatography using a SP-2560 flexible fused silica capillary column (100 m × 0.25 mm, inner diameter, 20 μm film thickness; Supelco, Inc., Bellefonte, PA USA) in a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector (Palo Alto, CA USA). The column temperature was programmed from 180° to 220°C at a rate of 1°C/min and then held for 20 min. Injector and detector temperatures were set at 250°C and the column head pressure was set at 15 PSI.

Experimental design and analysis

For the TBARS assay, the dose-effect of green tea epicatechins either as a mixture or an individual isomer on Cu^{+2}-mediated oxidation of human LDL was tested at the concentrations ranging from 0 to 40 μM. For the fatty acid analysis, the effect of individual jasmine tea epicatechins either as a GTP mixture or an individual isomer on Cu^{+2}-mediated oxidation of LDL polyunsaturated fatty acids was performed at the concentration of 5 μM. Data reported were expressed as mean ± SD of 6 to 8 samples. Differences between treatments and over time have been analyzed by a three-factor ANOVA, where factors were concentrations of jasmine tea epicatechins, type of individual epicatechin isomers, and the time of incubation.[23] Data for fatty acid analysis, Duncan's multiple range test was used to test the significant difference among the means of treatments.[24] This was performed by running data on the PC ANOVA software (PC ANOVA for the IBM personal computer, Version 1.1, 1985; IBM, Armonk, NY USA). Differences were considered to be significant when $P < 0.05$ or to be very significant when $P < 0.01$.

Table 1 Composition of jasmine green tea epicatechin isomers*

Polyphenol isomers	Absolute (g/100g tea)	Relative (% Total)
Epigallocatechin gallate (EGCG)	3.8 ± 0.1	51.2 ± 1.5
Epigallocatechin (EGC)	1.4 ± 0.1	18.7 ± 0.9
Epicatechin (EC)	0.9 ± 0.1	12.29 ± 0.6
Epicatechin gallate (ECG)	0.9 ± 0.1	11.78 ± 0.3
Caffeine	0.3 ± 0.1	4.3 ± 1.7
Others	0.1 ± 0.1	1.7 ± 0.5
Total	7.4 ± 0.1	100

*Data are expressed as means ± SD of $n = 6$ samples.

Results

The absolute and relative composition of jasmine tea GTP is shown in *Table 1*. These polyphenols present in jasmine tea were mainly epicatechin isomers including EGCG, EGC, EC, and ECG. The chemical structures of these epicatechin isomers are characterized by sharing a similar backbone with varying number and location of hydroxyl groups (*Figure 2*). HPLC analysis showed that the yield of jasmine tea GTP was 7.4 g/100 g dry jasmine tea leaves from six determinations. EGCG was the major isomer and accounted for 51.2%, followed by EGC (18.7%), EC (12.3%), and ECG (11.8%) in a decreasing order.

Effect of jasmine tea GTP extracts on production of TBARS was examined by incubating human LDL at the presence of 5 μM $CuSO_4$ as an oxidation initiator. The average molecular weight (MWt) of jasmine tea GTP extracts was calculated to be 388.1 based on the MWt of individual GTP isomers and their percentage in GTP extracts [*Table 1*; the average MWt = (MWt_{EGCG} × 51.2% +

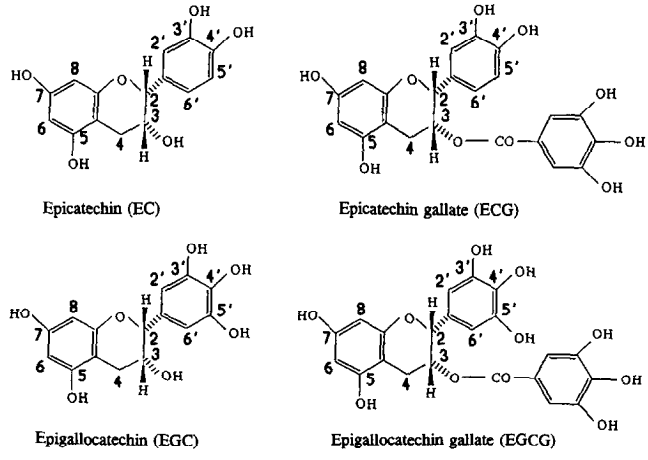

Figure 2 Chemical structures of (−) epigallocatechin (EGC), (−) epigallocatechin gallate (EGCG), (−) epicatechin (EC), and (−) epicatechin gallate (ECG).

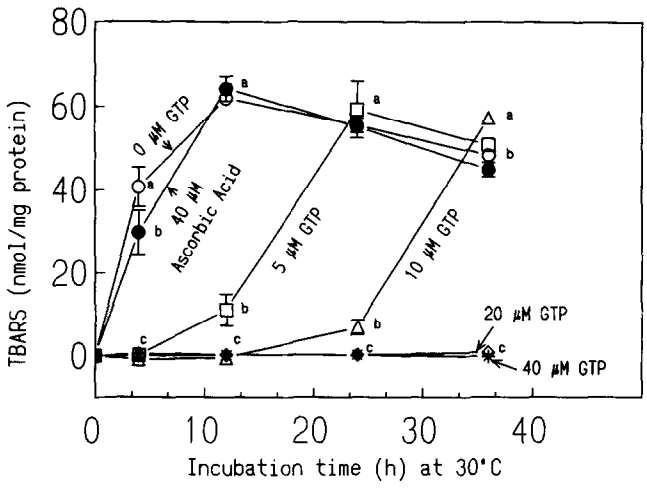

Figure 3 Inhibitory effect of jasmine green tea polyphenols (GTP) on production of thiobarbituric acid reactive substances (TBARS) in Cu^{+2}-mediated oxidation of human LDLs. Data are expressed as mean ± SD of n = 6 to 8 samples. ^{a,b,c}Means at the same time point with different letters differ very significantly ($P < 0.01$).

$MWt_{EGC} \times 18.7\% + MWt_{EC} \times 12.2\% + MWt_{ECG} \times 11.8\% + MWt_{caffeine} \times 4.3\%$)]. As shown in *Figure 3*, LDL was oxidized significantly within 4 hr at the absence of jasmine GTP extracts. Ascorbic acid at the concentrations of 5 to 40 μM (only data for 40 μM shown) demonstrated no or little protection to LDL from Cu^{+2}-catalyzed oxidation. In contrast, antioxidative activity of jasmine tea GTP extracts against LDL oxidation was dose-dependent at the concentrations ranging from 5 to 40 μM (P for trend = 0.01). To be specific, addition of 5 and 10 μM of jasmine tea GTP extracts extended the lag-time up to more than 12 and 24 hr, respectively (*Figure 3*). In the presence of 20 and 40 μM of jasmine tea GTP extracts, LDL did not show any oxidation throughout the period of 36 hr.

All four epicatechin isomers isolated from jasmine tea demonstrated a dose-dependent antioxidant activity in Cu^{+2}-promoted LDL oxidation (*Figure 4*, P for trend = 0.01). When LDL was incubated with 5 μM epicatechin isomers for 12 hr, EGCG and ECG were more effective than EGC and EC ($P < 0.01$). Under the same conditions, EC seemed to be more effective than EGC (*Figure 4*). For the samples with addition of 10 μM EGCG and ECG, production of TBARS was negligible throughout the experiment. When the samples with addition of 10 μM EC and EGC were incubated for 12 hr at 30°C, the former was more effective than the latter against LDL oxidation ($P < 0.01$). Similarly, EC was more effective than EGC at the concentration of 20 μM. To simplify the data, the following features could be generalized: (1) EGC and EC were less effective than their corresponding gallate derivatives, EGCG and ECG (*Figure 4*); and (2) EC was more effective than ECG against LDL-oxidation.

Fatty acid analysis revealed that polyunsaturated fatty acid moieties including docosahexaenoic (22:6,n-3), arachidonic (20:4,n-6), α-linolenic (18:3,n-3), and linoleic acid (18:2,n-6) were susceptible to oxidation when human LDL was incubated in the presence of 5 μM $CuSO_4$ for 4 to 12 hr (*Table 2*). The addition of 5 μM jasmine tea GTP or individual epicatechin isomers, however, significantly prevented the loss of these polyunsaturated fatty acids in human LDL incubated under the same conditions (*Table 2*). When human LDL was incubated for 12 hr, EGC seemed to be less protective than other three isomers against degradation of 22:6,n-3; 20:4,n-6; 18:3,n-3; and 18:2,n-6 in Cu^{+2}-mediated oxidation (*Table 2*).

Discussion

Jasmine tea is an excellent source of natural polyphenol antioxidants. The extraction method used in the present study could yield 7.4 g GTP out of 100 g dry tea leaves. These antioxidants are mainly epicatechin isomers including EGCG, EGC, EC, and ECG (*Table 1*). If a jasmine tea drinker has five cups of tea beverages per day, the total GTP intake can be estimated to be 740 mg GTP/day provided that each cup of tea beverage contains 2 g dry tea leaves.

The Zutphen Elderly Study by Hertog et al.[10] showed an inverse association between tea consumption and coronary heart disease mortality after adjustment for age, diet, and other risk factors including history of myocardial infarction, intake of total energy, intake of saturated fatty acids, physical activity, body-mass index, smoking, serum total cholesterol, and systolic blood pressure. The benefits of drinking tea may be attributed to the content of tea epicatechin isomers. This is probably because an assortment of compounds including superoxide anions, cholesterol oxide, hydroxyl radicals, and lipid peroxyl radicals may trigger LDL-oxidation in vivo and promote atherogenesis process. In contrast, GTP as dietary antioxidants may attenuate the progress of atherosclerosis and delay the autoxidation by inhibiting formation of free radicals, interrupting the propagation of the free radical chain reaction and protecting LDL from oxidative modification. In the present study, we have examined antioxidant activity of jasmine tea GTP as a mixture or individual epicatechin isomers on Cu^{+2}-promoted LDL oxidation. It clearly demonstrated that jasmine tea GTP and its individual epicatechin isomers strongly inhibit LDL-oxidation (*Figures 3* and *4*). The inhibitory effect of jasmine tea GTP and its epicatechin isomers on LDL-oxidation was even more stronger than ascorbic acid, which has been shown to act as an antioxidant against LDL-oxidation.[25]

The biochemical mechanism by which jasmine tea GTP and individual epicatechin isomers inhibit LDL oxidation remains unclear. Probably, they protect LDL from oxidative modification by either one or combination of following possibilities: (1) GTP or individual epicatechin isomers may function as a primary antioxidant by directly reducing the formation of free radicals mediated by Cu^{+2}; (2) they may spare, maintain and regenerate α-tocopherol and other antioxidants by donating a hydrogen to the α-tocopherol radicals; and (3) they function as chelators to inactivate Cu^{+2} and other ions involved in initiation of free radicals.

It was also interesting to observe that EC and EGC were less effective than their corresponding gallate derivatives, EGC and EGCG, as antioxidants against LDL oxidation (*Figure 4*). Perhaps, an additional gallate group increases total number of phenol hydroxyl groups. For one thing, it

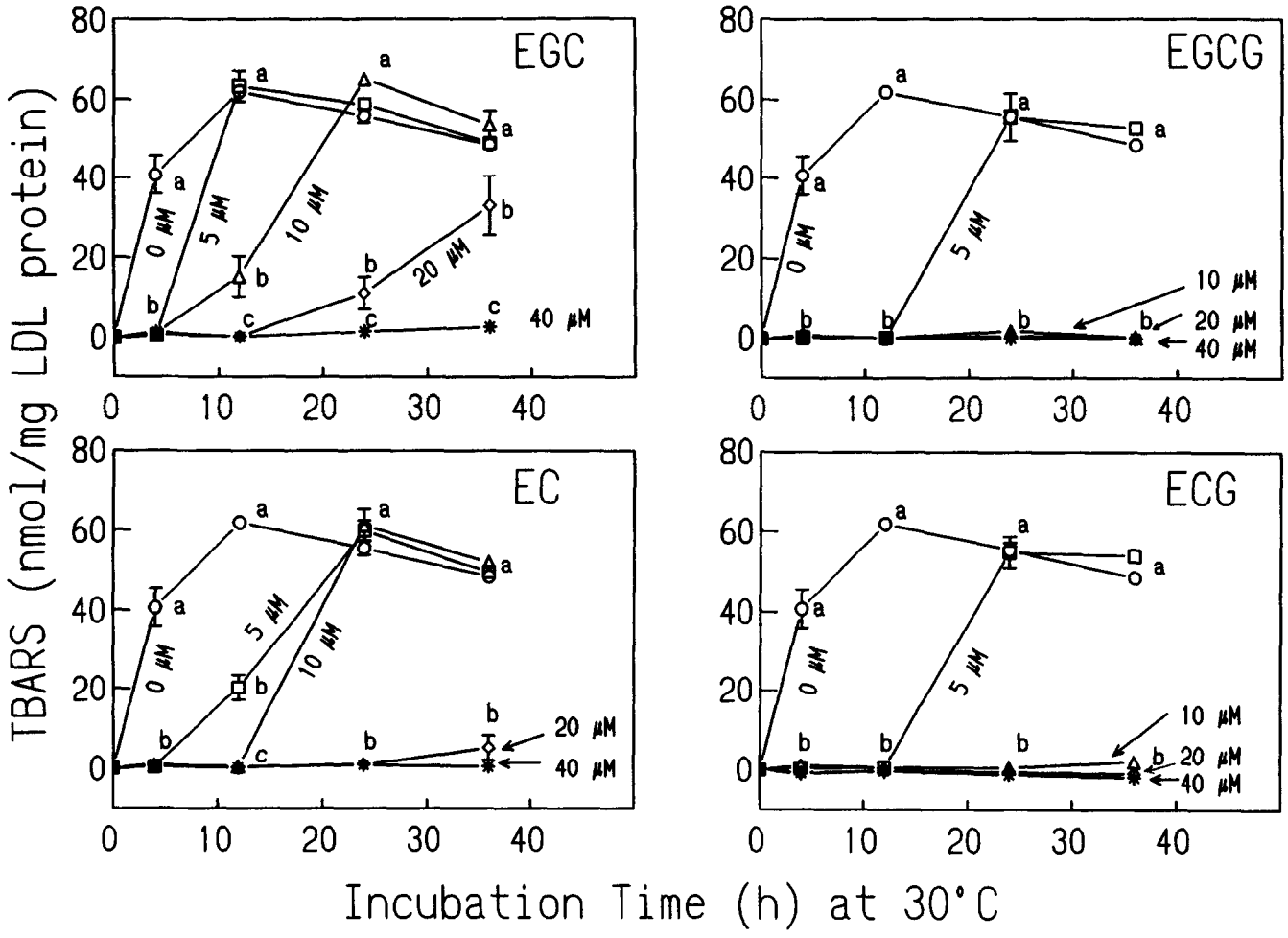

Figure 4 Inhibitory effect of jasmine green tea epicatechin isomers on production of thiobarbituric acid reactive substances (TBARS) in Cu^{+2}-mediated oxidation of human LDLs. Data are expressed as mean ± SD of $n = 8$ samples. EGC, (−) epigallocatechin; EGCG, (−) epigallocatechin gallate; EC, (−) epicatechin (EC); and ECG, (−) epicatechin gallate. [a,b,c]Means at the same time point with different letters differ very significantly ($P < 0.01$).

makes ECG and EGCG more vulnerable to donate a hydrogen than EC and EGC; for another, it makes ECG and EGCG more hydrophillic and therefore more chelating power to Cu^{+2}. This explanation may be only applicable to an emulsion system. In a nonemulsion system, the reversal was seen.[17] We have examined previously antioxidative activity of these four epicatechin isomers in frying canola oil and found that EC and EGC were more effective than their corresponding gallate derivative, ECG and EGCG, against oxidation of canola oil.[17] This is because an additional gallate group makes ECG and EGCG more hydrophillic, less soluble in oil, and therefore less effective against lipid oxidation in canola oil.

It seemed that EGCG and ECG had almost similar antioxidant activity under the present experimental conditions. However, EC was more effective than EGC against LDL oxidation. This was in agreement with the results of Miura et al.,[26] who showed that inhibitory effects of EC was stronger than EGC on LDL oxidation by measuring formation of diene. For the same reason, the antioxidant activity of epicatechin isomers may increase with number of hydroxyl groups, because of the vulnerable loss of a proton and stability of the free radical intermediate because of resonance delocalization.[27] On the other hand, the antioxidant activity of epicatechin isomers may decrease with number of total hydroxyl groups in an emulsion. This is because hydrophobicity decreases with number of hydroxyl group and, therefore, lesser amounts of epicatechin isomers are distributed into the lipid phase, where oxidation occurs.[28] By the same deduction, ECG has an additional hydroxyl group at position 5′ compared with EC (*Figure 2*). Thus, EGC was less effective than EC against LDL oxidation because lesser amounts of EGC than EC would be expected to distribute into lipid phase in LDL particle provided that EGC and EC had same concentrations in the incubation mixture.

The role of oxidized LDL in atherosclerosis remains controversial.[1–4] Oxidation of LDL is a free radical-mediated process. The polyunsaturated fatty acids in LDL are probably most susceptible to oxidation. The present study has demonstrated clearly that the polyunsaturated fatty acids in LDL could be protected from oxidation by green tea epicatechin isomers either alone or as a mixture. Besides

Table 2 Effects of jasmine tea polyphenol (GTP) extracts and individual epicatechin isomers (5 μM) on change in major fatty acids (μmol/g LDL protein) of human low-density lipoproteins (LDL) incubated at 30°C in a mixture containing 5 μM $CuSO_4$*

	16:0	16:1n-7	18:0	18:1n-9	18:2n-6	18:3n-3	20:4n-6	22:6n-3
Unoxidized LDL	493.5 ± 49.0^{a}	52.9 ± 4.8^{a}	159.7 ± 14.5^{a}	499.4 ± 40.9^{a}	881.6 ± 68.4^{a}	15.8 ± 9.7^{a}	153.9 ± 9.7^{a}	35.8 ± 4.2^{a}
			4 hours					
LDL + Cu^{+2}	490.0 ± 34.2^{a}	44.5 ± 5.5^{b}	144.8 ± 10.3^{b}	431.9 ± 20.0^{b}	199.4 ± 21.3^{c}	0	10.6 ± 1.0^{c}	0
LDL + Cu^{+2} + GTP	490.1 ± 52.3^{a}	52.9 ± 8.7^{a}	155.2 ± 9.0^{a}	512.3 ± 38.4^{a}	875.2 ± 59.4^{a}	15.5 ± 4.2^{ab}	154.2 ± 8.7^{a}	34.2 ± 5.2^{a}
LDL + Cu^{+2} + EGC	488.1 ± 22.3^{a}	55.8 ± 6.1^{a}	151.0 ± 8.7^{ab}	481.3 ± 14.2^{a}	835.8 ± 24.2^{a}	16.1 ± 1.9^{a}	142.6 ± 9.4^{a}	31.0 ± 4.8^{ab}
LDL + Cu^{+2} + EGCG	490.0 ± 39.4^{a}	56.5 ± 5.2^{a}	149.7 ± 9.7^{ab}	489.9 ± 29.4^{a}	842.9 ± 19.4^{a}	16.4 ± 1.9^{a}	144.8 ± 7.4^{a}	33.2 ± 4.7^{a}
LDL + Cu^{+2} + EC	488.1 ± 29.4^{a}	53.5 ± 4.5^{a}	153.2 ± 9.4^{a}	500.3 ± 18.7^{a}	863.5 ± 38.7^{a}	16.1 ± 1.0^{a}	149.4 ± 2.9^{a}	28.0 ± 5.8^{ab}
LDL + Cu^{+2} + ECG	497.1 ± 52.9^{a}	54.8 ± 4.8^{a}	153.2 ± 20.3^{ab}	494.5 ± 23.9^{a}	852.3 ± 19.7^{a}	16.2 ± 1.8^{a}	146.1 ± 9.4^{a}	32.6 ± 8.7^{a}
			12 hours					
LDL + Cu^{+2}	396.8 ± 33.9^{b}	30.3 ± 2.9^{c}	119.7 ± 9.4^{c}	279.4 ± 31.0^{c}	42.9 ± 10.9^{d}	0	0	0
LDL + Cu^{+2} + GTP	495.5 ± 31.3^{a}	53.2 ± 19.7^{a}	152.6 ± 9.4^{a}	508.7 ± 21.3^{a}	894.8 ± 79.3^{a}	12.6 ± 4.5^{b}	154.2 ± 8.7^{a}	36.5 ± 9.3^{a}
LDL + Cu^{+2} + EGC	491.6 ± 89.0^{a}	51.9 ± 8.4^{a}	143.5 ± 28.1^{b}	457.7 ± 71.3^{ab}	357.7 ± 14.8^{b}	3.2 ± 2.6^{c}	38.1 ± 10.3^{b}	6.5 ± 4.5^{c}
LDL + Cu^{+2} + EGCG	485.5 ± 48.7^{a}	53.5 ± 11.0^{a}	160.9 ± 16.5^{a}	496.1 ± 10.6^{a}	846.8 ± 89.4^{a}	15.8 ± 1.0^{a}	146.4 ± 8.7^{a}	33.5 ± 2.6^{a}
LDL + Cu^{+2} + EC	476.5 ± 49.7^{a}	54.8 ± 10.6^{a}	144.8 ± 7.4^{b}	481.9 ± 8.7^{a}	830.3 ± 33.2^{a}	14.5 ± 0.9^{a}	132.6 ± 10.3^{a}	25.5 ± 3.9^{b}
LDL + Cu^{++} + ECG	479.0 ± 52.9^{a}	56.4 ± 10.0^{a}	154.5 ± 10.9^{a}	474.5 ± 40.3^{a}	826.8 ± 72.7^{a}	15.2 ± 1.9^{a}	149.0 ± 11.3^{a}	31.6 ± 6.1^{ab}

*Data were expressed as mean ± SD of $n = 6$ to 8 samples.
^{a,b,c,d}Means at the same column with different superscript letters differ very significantly ($P < 0.01$).
EGC, (−) epigallocatechin; EGCG, (−) epigallocatechin gallate; EC, (−) epicatechin; and ECG, (−) epicatechin gallate.

polyunsaturated fatty acids, the protein moiety and cholesterol in LDL may also be targets of free radicals.[29,30]

The green tea drinkers may have lower plasma cholesterol and therefore may have lower risk in coronary heart disease mortality.[10–12] In the study by Imai and Nakachi,[11] not only plasma total cholesterol but also plasma triacylglycerols were inversely correlated to consumption of green tea. The current research in this laboratory has shown that jasmine tea GTP as a mixture significantly decreases plasma total cholesterol and triacylglycerols in hamster (Chan, P.T. et al, unpublished data). The biochemical actions of these epicatechin isomers involved in hypocholesterolemic effect remain poorly understood. Probably, they may reduce plasma cholesterol by either one or combination of the following mechanisms including decreasing activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (a regulatory enzyme in cholesterol biosynthesis) and decreasing absorption and reabsorption of dietary cholesterol and bile acids.[31]

In conclusion, the present results indicate that jasmine tea GTP as a mixture would protect LDL from oxidative modification in vitro. If tea consumption in humans is associated with a significant decrease in cardiovascular disease, part of the mechanisms may involve protection for LDL particles against oxidative modification and reduction in plasma LDL cholesterol.

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