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Antioxidative Activity of Green Tea Polyphenol in Cholesterol-Fed Rats

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This study investigated the effects of green tea polyphenol on the serum antioxidative activity and cholesterol levels of cholesterol-fed rats and compared them with those of probucol, an antioxidant hypocholesterolemic agent. To evaluate the antioxidative activity, the susceptibility to oxidative modification of low-density lipoprotein (LDL) isolated from the serum of cholesterol-fed rats was measured, as was the serum antioxidative activity using the spontaneous autoxidation system of brain homogenate. Administration of green tea polyphenol effectively inhibited LDL oxidation and elevated serum antioxidative activity to the same degree as probucol. However, higher amounts of polyphenol than probucol needed to be administered to reduce the total, free, and LDL cholesterol levels. Furthermore, green tea polyphenol increased the levels of high-density lipoprotein (HDL) cholesterol, leading to dose-dependent improvement of the atherogenic index, an effect that was not seen with probucol. Thus, green tea polyphenol may exert an antiatherosclerotic action by virtue of its antioxidant properties and by increasing HDL cholesterol levels.

KEYWORDS: Green tea; polyphenol; antioxidant

INTRODUCTION

Recently, the physiological effects of polyphenol-rich foods, such as fruits, vegetables, and beverages including fruit juices, wine, tea, coffee, and chocolate, have been receiving a lot of attention as dietary sources of antioxidants that are valuable for human health. Many epidemiological studies have strongly suggested there is a correlation between intake of polyphenol-rich foods and low mortality due to coronary heart disease (CHD) (*1*−*3*). CHD, such as myocardial infarction and ischemic stroke, which is closely related to atherosclerosis, is a major cause of death in advanced countries. Therefore, it is worth studying the impact of the daily consumption of polyphenol-rich foods and the extent of the effects of such foods on atherosclerosis.

Recent studies have implicated oxidative damage as an important etiologic factor in atherosclerosis. Especially, according to the oxidative stress theory, oxidative modification of low-density lipoprotein (LDL) is thought to play a key role in the development of atherosclerosis (*4*−*6*). Therefore, inhibiting this process is considered to be an important therapeutic approach. Indeed, vitamin E and probucol have been reported to prevent LDL oxidation and delay the development of atherosclerotic plaques in animal models (*7*, *8*), suggesting the effectiveness of antioxidants for the treatment and prevention of atherosclerosis.

Green tea is a widely consumed beverage, which mainly contains low molecular weight polyphenols belonging to the flavan-3-ol class of flavonoids. In animal experiments, green tea polyphenol was demonstrated to reduce serum cholesterol levels, the elevation of which is one of the risk factors for atherosclerosis (*9*). Furthermore, green tea polyphenol is known to be an excellent antioxidant that directly scavenges free radicals and inhibits lipid peroxide formation (*10*−*13*). Green tea, owing to its antioxidative activity, has also been reported to inhibit hypertension, mutagenesis, and tumorigenesis and to protect against renal diseases in several experimental systems in in vitro and in vivo (*14*−*17*).

Therefore, we performed a study in cholesterol-fed rats to examine the effects of green tea polyphenol on serum antioxidative activity and cholesterol levels and compare them with those of probucol, a frequently used hypocholesterolemic agent with antioxidant activity, to further our understanding of the preventive effects of this compound on CHD.

MATERIALS AND METHODS

Green Tea Polyphenol. The green tea polyphenol mixture employed was Sunphenon (Taiyo Kagaku Co., Yokkaichi, Japan), which was prepared from a hot-water extract of green tea with a recovery rate of ∼9.6%, by weight, of the original pulverized Japanese green tea, as reported previously (*18*). It was composed mainly of (−)-epigallocatechin 3-*O*-gallate (18.0%), (−)-gallocatechin 3-*O*-gallate (11.6%), (−)-epicatechin 3-*O*-gallate (4.6%), (−)-epigallocatechin (15.0%), (+)-gallocatechin (14.8%), (−)-epicatechin (7.0%), and (+)-catechin (3.5%).

Animals and Treatment. The *Guiding Principles for the Care and Use of Laboratory Animals* and *Guidelines for Animal Experimentation*

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Table 1. Effect of Green Tea Polyphenol and Probucol on LDL Peroxidation

group	TBA-RS[a] (nmol/mg of LDL protein)
normal rats	3.42 ± 0.12
cholesterol-fed rats	
control	12.08 ± 0.30 a
0.1% polyphenol	8.29 ± 0.49 ab
0.5% polyphenol	7.33 ± 0.37 ab
2.5% polyphenol	5.07 ± 0.03 ab
0.1% probucol	5.28 ± 0.17 ab

[a] Statistical significance: a, $p < 0.001$ vs normal rats; b, $p < 0.001$ vs cholesterol-fed control rats.

Table 2. Effect of Green Tea Polyphenol and Probucol on Serum Antioxidant Activity

group	antioxidant activity[a] (%)
normal rats	44.5 ± 2.5
cholesterol-fed rats	
control	33.2 ± 3.0 a
0.1% polyphenol	41.8 ± 4.2 b
0.5% polyphenol	43.1 ± 4.7 b
2.5% polyphenol	43.8 ± 4.3 c
0.1% probucol	49.2 ± 6.2 d

[a] Statistical significance: a, $p < 0.01$ vs normal rats; b, $p < 0.05$; c, $p < 0.01$; d, $p < 0.001$ vs cholesterol-fed control rats.

approved by the Japan Pharmacological Society and Japanese Association for Laboratory Animal Science, respectively, were followed in these experiments. Male Wistar rats (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan) weighing 160−170 g were kept in an automatically controlled room (the room temperature was ∼23 °C, and the humidity was ∼60%) with a conventional lighting regimen with a dark night. They were divided into six groups: one was fed a basal diet, and the others were fed an experimental high-cholesterol diet containing 1% cholesterol and 10% coconut oil. Green tea polyphenol and probucol were administered as dietary supplements in the high-cholesterol diet as follows: group 1, basal diet (normal group); group 2, high-cholesterol diet (control group); group 3, high-cholesterol diet containing 0.1% polyphenol; group 4, high-cholesterol diet containing 0.5% polyphenol; group 5, high-cholesterol diet containing 2.5% polyphenol; group 6, high-cholesterol diet containing 0.1% probucol. During the experimental period, consumption of diet was kept at the same amount (∼13 g/rat). Polyphenol intake was estimated at 13, 65, and 325 mg for the 0.1, 0.5, and 2.5% polyphenol groups, respectively, and probucol intake at 13 mg. After 5 weeks, the rats were sacrificed by decapitation and blood samples were taken. The serum obtained by centrifugation was used for LDL separation and determination of chemical parameters.

LDL Preparation and Oxidation. LDL was isolated from serum by density-gradient ultracentrifugation, as described previously (*19*), and dialysis against two changes of 0.15 M NaCl (pH 7.4) for 48 h at 4 °C. Before oxidation, LDL was diluted with 0.15 M NaCl (pH 7.4) to a concentration of 300 μg of protein/mL. LDL protein levels were determined according to the method of Lowry et al. (*20*). Oxidation of LDL was carried out by incubation with freshly prepared 20 μM $CuSO_4$ at 37 °C for 4 h in a shaking water bath, immediately after which the extent of lipid peroxidation was determined by measuring the amount of thiobarbituric acid reactive substances (TBA-RS) formed (*21*).

Determination of Serum Antioxidative Activity. According to the method of Kuroda et al. (*22*), 20 μL of serum was added to 2 mL of brain homogenate and incubated at 37 °C for 60 min. The amounts of lipid peroxide in the zero-time and 60-min aliquots were determined by measuring the TBA-RS levels (*23*). The antioxidative activity of each serum sample is expressed as percentage inhibition relative to the amount of TBA-RS formed in the above reaction mixture without serum.

Determination of Serum Cholesterol Levels. Total and free cholesterol levels were determined using commercial assay kits (Cholesterol E-Test Wako and Free Cholesterol E-Test Wako obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan). The LDL and high-density lipoprotein (HDL) cholesterol levels were determined according to the method of Noma et al. (*24*, *25*). The atherogenic index (AI) was calculated as follows: AI = (total cholesterol − HDL cholesterol)/HDL cholesterol.

Statistics. Data are presented as means ± SE. Differences among groups were analyzed by Dunnett's test, and those at $p < 0.05$ were considered to be significant.

RESULTS

LDL Oxidation. Table 1 shows the peroxidation levels of LDL isolated from the blood of normal and cholesterol-fed rats. LDL was oxidized by Cu^{2+}, resulting in the formation of peroxides. Administration of green tea polyphenol markedly inhibited this peroxidation reaction: even the lowest dose (0.1%) reduced the level by 31% to 8.29 nmol/mg of LDL protein compared with the control value, 12.08 nmol/mg of LDL protein. Similarly, 0.5% green tea polyphenol reduced the level by 39% to 7.33 nmol/mg of LDL protein, and stronger inhibition, 58%, was observed in the 2.5% polyphenol group, nearly as high as that in the 0.1% probucol group.

Antioxidative Activity in Serum. The antioxidative activity value of the control group was 33.2%, significantly lower than that of the normal group, 44.5%, as shown in **Table 2**. Administration of green tea polyphenol increased the antioxidant activity, which was increased more, to 49.2%, in the probucol administration group.

Serum Cholesterol Levels and AI. The serum cholesterol level and AI results are summarized in **Table 3**. The rats fed the high-cholesterol diet had significantly higher total, free, and LDL cholesterol levels and significantly lower HDL cholesterol levels than the rats fed the basal diet. Total cholesterol was reduced significantly by 11 and 21% after administration of 0.5 and 2.5% green tea polyphenol, respectively, but 0.1% polyphenol did not change it. Administration of 0.1% probucol significantly reduced it to the same extent as 2.5% polyphenol. The free cholesterol levels were significantly reduced by 31% in the 2.5% polyphenol group and by 27% in the 0.1% probucol group, whereas they were not affected by 0.1 and 0.5% polyphenol. A similar trend was found for LDL cholesterol levels; they were reduced by 17 and 11% in the 2.5% polyphenol and 0.1% probucol groups, respectively. In addition, green tea polyphenol significantly increased HDL cholesterol levels at all three dose groups, as shown in **Table 3**. However, probucol did not change the HDL cholesterol level. The AI was significantly reduced in a dose-dependent manner by administration of green tea polyphenol, but not probucol, reflecting the HDL cholesterol levels.

DISCUSSION

Oxidative damage plays an important role in the pathogenesis of atherosclerosis. It is widely accepted that modification of LDL is necessary for the scavenger receptor uptake that leads to cellular cholesterol accumulation and foam cell formation. Kita et al. demonstrated that the antioxidant probucol inhibited peroxidation of LDL and attenuated development of atherosclerosis in Watanabe heritable hyperlipidemic rabbits (*7*). In addition, oxidative modification of LDL has been detected in atherosclerotic lesions by immunohistochemistry using a monoclonal antibody (*26*). From these findings, there is no doubt that LDL undergoes oxidative modification in vivo and that inhibition of LDL oxidation is important for the attenuation of

Table 3. Effect of Green Tea Polyphenol and Probucol on Serum Cholesterol Levels and AI

group	total cholesterol[a] (mg/dL)	free cholesterol[a] (mg/dL)	LDL cholesterol[a] (mg/dL)	HDL cholesterol[a] (mg/dL)	AI[a]
normal rats	64.1 ± 2.0	18.2 ± 1.1	25.7 ± 1.7	37.1 ± 2.4	0.73 ± 0.22
cholesterol-fed rats					
control	160.5 ± 5.4 a	28.7 ± 1.1 a	135.5 ± 4.4 a	11.8 ± 0.4 a	13.72 ± 0.89 a
0.1% polyphenol	158.7 ± 9.7 a	28.9 ± 1.3 a	136.1 ± 7.9 a	15.0 ± 0.9 ad	10.22 ± 0.90 ad
0.5% polyphenol	142.3 ± 7.6 ac	27.0 ± 1.0 a	133.8 ± 7.0 a	15.0 ± 1.1 ad	9.64 ± 0.86 ad
2.5% polyphenol	127.0 ± 7.0 ad	19.9 ± 1.0 ad	112.7 ± 4.8 ad	13.5 ± 0.9 ab	8.92 ± 0.89 ad
0.1% probucol	130.6 ± 7.5 ad	21.0 ± 1.3 ad	121.0 ± 8.3 ac	10.9 ± 0.7 a	12.63 ± 1.30 a

[a] Statistical significance: a, $p < 0.001$ vs normal rats; b, $p < 0.05$; c, $p < 0.01$; d, $p < 0.001$ vs cholesterol-fed control rats.

atherosclerotic changes. Oxidized LDL contributes to various atherosclerotic stages, not only foam cell formation via scavenger receptor uptake but also direct chemotactic activity toward monocytes, smooth muscle cells, and T-lymphocytes, inhibition of biological activity and production of endothelium-derived nitric oxide (NO), and induction of cytotoxicity leading to endothelial injury (*4*, *27*). In the present study, we found that administration of 0.1% green tea polyphenol (the lowest dose given) markedly reduced the susceptibility of LDL to oxidation, although probucol at the same dose had a stronger effect.

LDL is oxidized by metal ions or arterial wall cells including endothelial cells, smooth muscle cells, and macrophages (*28*). It was concluded that superoxide radicals (O_2^-) are produced at increased rates in hypercholesterolemic vessels (*29*) and that this increased O_2^- may inactivate NO and provide a source for other oxygen radicals, including peroxynitrite and the hydroxyl radical. Thus, a defense system against free radicals in serum in contact with arterial walls is important to prevent LDL oxidation. The serum contains a number of antioxidant systems including enzymes, such as superoxide dismutase, albumin, ceruloplasmin, transferrin, ascorbic acid, α-tocopherol, β-carotene, glutathione, and others. In this study, we attempted to measure the total antioxidant activity of serum using the system of spontaneous autoxidation of brain homogenate, which contains a large amount of polyunsaturated fatty acid (*22*). We found that the antioxidative activity of serum was elevated in the green tea polyphenol and probucol groups, suggesting that the endogenous antioxidant status became stronger. These findings suggest that administration of green tea polyphenol inhibits LDL oxidation, which is a key step in atherosclerotic progression due to elevated endogenous antioxidative activity and the subsequent development of atherosclerotic lesions. The antioxidative activity of green tea polyphenol was found to be significant, although probucol had stronger activity.

Our data show that 2.5% polyphenol administration reduced serum levels of all types of cholesterol except HDL cholesterol, and its effects were almost equivalent to those after 0.1% probucol administration. On a weight basis, polyphenol intake was 25 times higher than probucol intake. We found that fecal excretion of total cholesterol increased (data not shown), supporting other findings that green tea polyphenol increased fecal excretion of cholesterol and bile acid (*9*, *30*) and reduced cholesterol absorption from the intestine (*31*). Therefore, we think that green tea polyphenol reduced serum total cholesterol levels by increasing fecal excretion of cholesterol. Our results showed that an extremely large amount of green tea polyphenol has to be administered to exert this effect. However, the HDL cholesterol level was increased by green tea polyphenol, with even the lowest dose (0.1%) inducing a significant 27% increase, whereas it was not changed by probucol. Administration of green tea polyphenol dose-dependently reduced the AI, which is commonly used as an index of risk for CHD, mainly by increasing the HDL cholesterol level rather than reducing the total cholesterol level. Recent studies showed that HDL promotes the reverse cholesterol transport pathway, in which HDL induces efflux of excess accumulated cellular cholesterol and prevents the generation of an oxidatively modified LDL (*32*, *33*). The studies on cholesterol-fed rabbits carried out by Badimon et al. suggested that it might be possible not only to inhibit progression but also to reduce established atherosclerotic lesions by HDL administration (*34*, *35*). Thus, our results suggest that green tea polyphenol has beneficial effects, such as the promotion of efflux of cholesterol accumulated in cells and the inhibition of LDL oxidation in vivo due to increasing serum concentrations of HDL. However, probucol is not expected to have this effect.

In summary, scientific interest in polyphenol-rich foods as a dietary source of antioxidants has increased in response to the recognition of the importance of oxidative damage in the pathogenesis of many diseases. Our present investigation provides information that explains, at least in part, the potential benefits of dietary polyphenols in patients with CHD. Although further research is required to establish whether green tea polyphenol attenuates the development of atherosclerotic lesions in vivo, our results suggest that this compound has beneficial effects in attenuating the atherosclerotic process due to its antioxidative activity and by increasing HDL cholesterol levels. In addition, it has a hypocholesterolemic effect, although this is weaker than that of the positive control probucol.

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