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Heat-Epimerized Tea Catechins Rich in Gallocatechin Gallate and Catechin Gallate Are More Effective To Inhibit Cholesterol Absorption than Tea Catechins Rich in Epigallocatechin Gallate and Epicatechin Gallate

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It has been known that tea catechins, (−)-epicatechin (**1**), (−)-epigallocatechin (**2**), (−)-epicatechin gallate (**3**), and (−)-epigallocatechin gallate (**4**) are epimerized to(−)-catechin (**5**), (−)-gallocatechin (**6**), (−)-catechin gallate (**7**), and (−)-gallocatechin gallate (**8**), respectively, during retort pasteurization. We previously reported that tea catechins, mainly composed of **3** and **4**, effectively inhibit cholesterol absorption in rats. In this study, the effect of heat-epimerized catechins on cholesterol absorption was compared with tea catechins. Both tea catechins and heat-epimerized catechins lowered lymphatic recovery of cholesterol in rats cannulated in the thoracic duct and epimerized catechins were more effective than tea catechins. The effect of purified catechins on micellar solubility of cholesterol was examined in an in vitro study. The addition of gallate esters of catechins reduced micellar solubility of cholesterol by precipitating cholesterol from bile salt micelles. Compounds **7** and **8** were more effective to precipitate cholesterol than **3** and **4**, respectively. These observations strongly suggest that heat-epimerized catechins may be more hypocholesterolemic than tea catechins.

KEYWORDS: Catechin gallate; cholesterol absorption; epicatechin gallate; epigallocatechin gallate; gallocatechin gallate; rats

INTRODUCTION

Green tea catechins (**Figure 1**), major components of which are (−)-epicatechin (**1**), (−)-epigallocatechin (**2**), (−)-epicatechin gallate (**3**), and (−)-epigallocatechin gallate (**4**) have been reported to have various physiological functions, such as antivirus (*1*), antioxidative (*2*, *3*), antimutagenic (*4*, *5*), anticarcinogenic (*6*), and antiobesity (*7*) activities. A hypocholesterolemic activity of catechins has been also reported in experimental animals (*8*−*11*). Our previous study showed a mechanism by which **3** and **4** effectively eliminated cholesterol from bile salt micelles and reduced intestinal absorption of cholesterol in lymph-cannulated rats (*12*).

Catechins are contained in various tea products such as green tea, oolong tea, and black tea (*13*). Consumption of canned and bottled tea drinks is increasing in Asian countries, in particular in Japan. These products are autoclaved for pasteurization generally at 120 °C for several minutes. It has been known that during the pasteurization, considerable amounts (around 50%) of catechins are epimerized at the 2-position, and (−)-catechin (**5**), (−)-gallocatechin (**6**), (−)-catechin gallate (**7**), and (−)-gallocatechin gallate (**8**) are formed (*13*, *14*). However, studies on physiological effects of heat-epimerized catechins are scarce (*15*), and no studies have been done regarding whether epimerized catechins may cause inhibitory effects on cholesterol absorption as in the case of green tea catechins. In this study, the effect of heat-epimerized catechins on lymphatic absorption and micellar solubility of cholesterol was compared with green tea catechins in rats and in vitro.

MATERIALS AND METHODS

Compounds **1**, **2**, and **5** were obtained from Wako Pure Chemicals, Osaka, Japan. Compounds **6**, **7**, and **8** were obtained from Nagara Science, Gifu, Japan. Compounds **3** and **4** were obtained from Kurita Water Industries, Tokyo, Japan. Purities of all of these catechins were >98%. A mixture of green tea catechins was THEA-FLAN 90S provided from Itoen Ltd, Shizuoka, Japan. Heat-epimerized catechins were prepared by autoclaving the catechin mixture at 120 °C for 5 min. The composition of catechins in green tea catechins and heat-epimerized catechins are shown in **Table 1**. Epimerized catechins prepared from green tea catechins contained increased amounts of **7**

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Table 1. Composition of Green Tea Catechins and Heat-Epimerized Catechins (%)

	(–)-epigallocatechin gallate (**4**)	(–)-gallocatechin gallate (**8**)	(–)-epicatechin gallate (**3**)	(–)-catechin gallate (**7**)	total
green tea catechins	49.9	4.09	17.1	1.15	72.3
heat-epimerized catechins	20.9	24.1	7.29	7.77	60.1

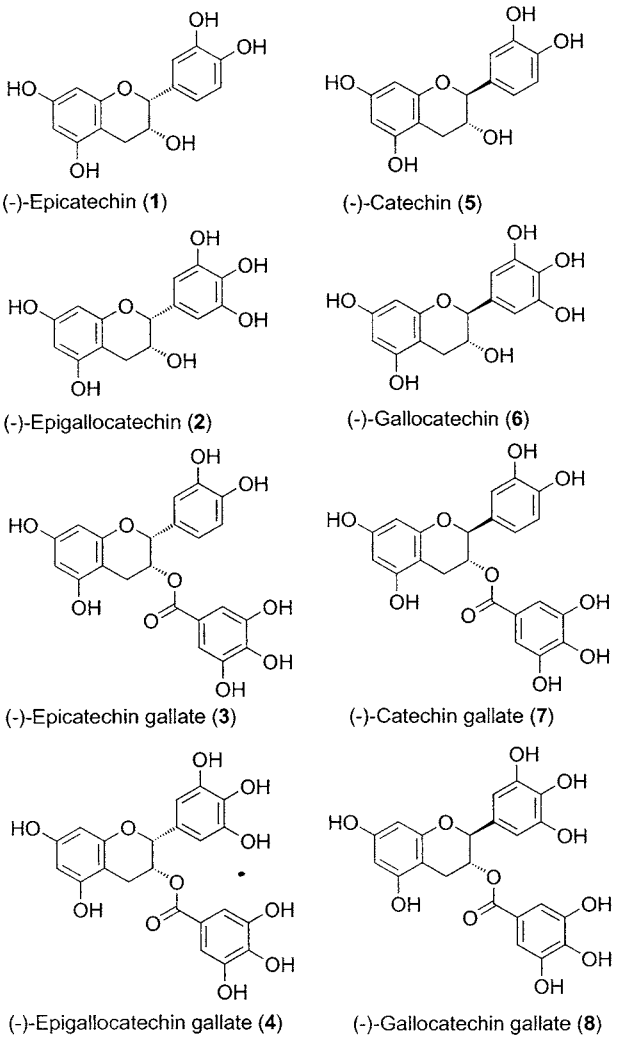

Figure 1. Chemical structures of various tea catechins and heat-epimerized catechins.

and **8**. 4-^{14}C-Cholesterol (55 mCi/mmol) was purchased from Amersham Pharmacia Biotech, Tokyo, Japan.

Studies on Lymphatic Recovery of ^{14}C-Cholesterol in Rats Cannulated in the Thoracic Duct. Eight-week old male SD rats were fed a commercial chow for 1 week until the operation. The left thoracic lymphatic duct cephalad to the cisterna chyli of these rats was cannulated as described previously (*16*). A second indwelling catheter was placed in the stomach for administration of a test emulsion. After surgery, the animals were placed in restraining cages and intragastrically given a continuous infusion of a solution containing 139 mM glucose and 85 mM NaCl at a rate of 3.4 mL/h until the end of the experiment. The same solution was given as drinking water. The next morning, animals with a constant lymph flow rate were administered 3 mL of a test emulsion containing ^{14}C-cholesterol with or without catechin preparations. The test emulsion (3 mL) contained 200 mg of sodium taurocholate (Nacalai tesque, Kyoto, Japan), 50 mg of fatty-acid-free bovine albumin fraction V (BSA, Bayer Corp. IL), 200 mg of triolein (Sigma, St Louis, MO), 37 kBq of ^{14}C-cholesterol and 25 mg of cholesterol. The mixture was emulsified by sonication. When tea catechins and heat-epimerized catechins were administered, these catechins were added in the emulsion at 100 mg and 120 mg in 3 mL, respectively. Because the content of catechins (**3**, **4**, **7**, and **8**) was decreased in heat-epimerized catechins, the amount of catechins was adjusted to the same levels between tea catechins and epimerized catechins. The molar ratios of total catechins to cholesterol in lipid emulsions were about 2.5. Lymph was collected in ice-chilled tubes containing EDTA, and the radioactivity was measured. All animal studies were carried out under the guidelines for animal experiments of the Faculty of Agriculture, Graduate School Kyushu University and Law 105 and Notification 6 of the government of Japan.

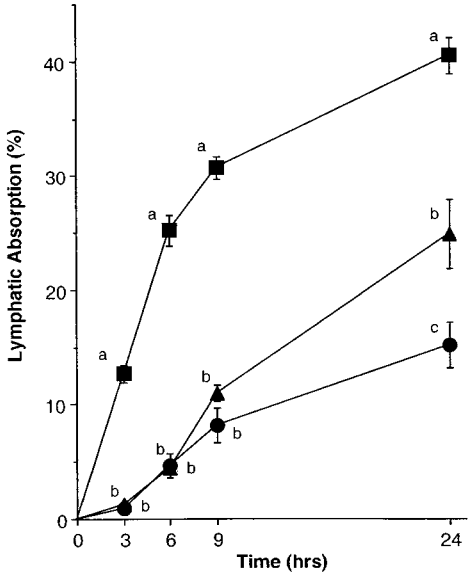

Figure 2. Effect of green tea catechins and heat-epimerized catechins on lymphatic absorption of [4-^{14}C] cholesterol in rats administered intragastrically fat emulsions. ■, Control group; ▲, tea catechin group; ●, heat-epimerized catechin group. Data are means ± SE of 6 or 7 rats. Means not sharing a common superscript letter are significantly different at $P < 0.05$.

Studies on Micellar Solubility of Cholesterol. The effect of various purified catechins on micellar solubility of cholesterol was examined according to our previous study (*12*). A bile salt micellar solution containing 6.6 mM sodium taurocholate, 0.6 mM egg yolk phosphatidylcholine, 0.5 mM cholesterol, 132 mM NaCl, and 15 mM sodium phosphate at pH 6.8 was prepared by sonication and kept at 37 °C for more than 24 h. The 100-μL solution of purified catechins kept at 37 °C were added to the 3-mL micellar solution. The amounts of catechins added were adjusted to 1 and 2 mM. The molar ratios of each catechin to cholesterol were 2 and 4. The mixture was incubated for 1 h at 37 °C. The solution was filtered using a 200 nm Whatman GD/X filter, and the filtrate obtained was subjected to cholesterol analysis by GC using an SPB-1 column (Supelco, PA). The concentration of bile acids in micelles was measured enzymatically using hydroxysteroid dehydrogenase (Sigma) (*17*).

Statistical Analysis. Data were analyzed by Bonferroni/Dann test to evaluate the significant difference between a pair of means.

RESULTS

Effect of Green Tea Catechins and Heat-Epimerized Catechins on Lymphatic Absorption of Cholesterol in Thoracic Duct Cannulated Rats. Lymph flow rates were

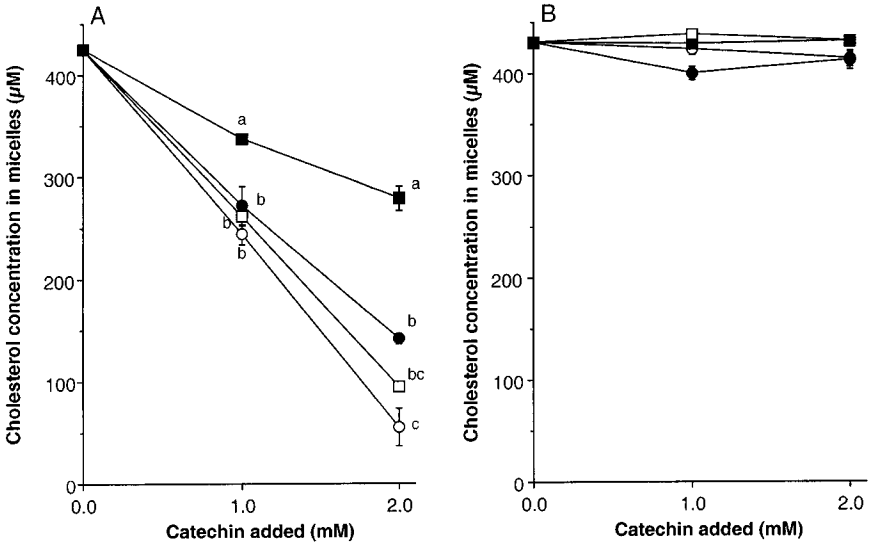

Figure 3. Effect of purified catechins on micellar solubility of cholesterol in vitro. A. Purified (–)-epicatechin gallate (**3**, ■), (–)-epigallocatechin gallate (**4**, ●), (–)-catechin gallate (**7**, □), or (–)-gallocatechin gallate (**8**, ○); B. Purified (–)-epicatechin (**1**, ■), (–)-epigallocatechin (**2**, ●), (–)-catechin (**5**, □), or (–)-gallocatechin (**6**, ○). Data are means ± SE of triplicate determinations. Values not sharing a common letter are significantly different at $P < 0.05$.

linear, and no difference was observed among the control, green tea catechin, and heat-epimerized catechin groups (175 ± 15, 165 ± 16, and 154 ± 17 mL/24 h, respectively). The administration of catechins effectively and significantly lowered lymphatic recovery of cholesterol compared to the control group during 24 h lymph collection (**Figure 2**). Epimerized catechins were more effective in lowering cholesterol absorption than tea catechins. The difference was significant. Lymphatic recovery of ^{14}C-cholesterol was 40.5 ± 1.6, 24.9 ± 3.0, and 15.2 ± 2.0% during 24 h lymph collection in the control, green tea catechin, and heat-epimerized catechin groups, respectively.

Effect of Purified Catechins on Micellar Solubility of Cholesterol in Vitro. All of the gallate esters of catechins reduced micellar solubility of cholesterol in a dose-dependent manner (**Figure 3A**). Differences in the effect of catechins on micellar solubility of cholesterol were observed in the addition at 2 mM concentration. The concentration of micellar cholesterol in the addition of **8** was lowest, and it was significantly lower than that in the addition of **3** and **4**. Compound **7** also effectively reduced micellar cholesterol, and the effect was almost comparable to **8**. In contrast to the gallate esters of catechins, all of the free catechins examined did not reduce micellar solubility of cholesterol (**Figure 3B**).

The concentration of bile acid in micelles was not influenced by the addition of any catechins (data not shown).

DISCUSSION

We previously showed that green tea catechins rich in **3** and **4** strongly inhibited lymphatic absorption of cholesterol in rats, and they precipitated cholesterol from bile salt micellar solution in vitro (*12*). The present study clearly showed that heat-epimerized catechins rich in **7** and **8** were more effective to reduce lymphatic absorption of cholesterol than green tea catechins rich in **3** and **4** in rats. Because heat-epimerized catechins used in this study contained a higher amount of unknown compounds than green tea catechins, as shown in **Table 1**, there is a possibility that unknown compounds can be effective to lower micellar solubility of cholesterol. This possibility cannot be disproved. However, according to our in vitro study, because purified **7** and **8** were more effective to reduce micellar cholesterol concentration than purified **3** and **4**, respectively, a more predominant lowering effect of epimerized catechins on cholesterol absorption can be ascribed to effective inhibition of micellar solubility of cholesterol by **7** and **8**. Although various physiological functions of tea catechins have been reported in many investigations, this study showed for the first time an inhibitory effect of heat-epimerized catechins on cholesterol absorption. In this study, the effect of heat-epimerized catechins on exogenously cholesterol absorption was examined. Endogenous cholesterol is also secreted to intestinal lumen as the bile in a bile salt micellar form. Therefore, our results suggest that heat-epimerized catechins may be more effective to inhibit the reabsorption of endogenous cholesterol than green tea catechins.

Several studies showed that dietary tea (*10*) and tea catechins (*8*, *9*, *11*), lowered plasma cholesterol concentration in experimental animals fed a high-cholesterol diet. Muramatsu et al. (*8*) observed that the feeding of green tea catechins increased fecal excretion of cholesterol in rats. This observation suggests that tea catechins reduce plasma cholesterol concentration by inhibiting cholesterol absorption. Although a hypocholesterolemic effect of heat-epimerized catechins has not been examined in experimental animals, we recently reported that when 450 mg/day of a mixture of tea catechins containing more than 50% of heat-epimerized catechins were given to mildly hypercholesterolemic subjects as a drink for 8 weeks, plasma cholesterol concentration was significantly lowered (*18*). Because there is actually no precise human study on hypocholesterolemic effect of tea catechins, differences in the effect on plasma cholesterol concentration between tea catechins and heat-epimerized catechins are not clear. However, our results strongly suggest that heat-epimerized catechins may be more effective to lower plasma cholesterol concentration than tea catechins.

When **3**, **4**, **7,** and **8** were added to bile salt micellar solutions, the solutions became turbid, and precipitates were produced. We previously showed that **4** coprecipitated with micellar cholesterol in a similar experimental condition (*12*). Although we did not show the coprecipitation of **7** and **8** with cholesterol in the present study, our results suggest that **7** and **8** can coprecipitate with cholesterol more effectively than can **3** and **4**.

The mode of association between gallic acid esters of catechins and cholesterol has never been understood. Kajiya et al. (*19*) showed the existence of a hydrophobic domain in gallate esters of catechins, but not in free catechins. They also showed that the gallate esters have a higher affinity for hydrophobic lipid bilayers than do free catechins. We speculate that gallate esters of catechins can be hydrophobically bound to cholesterol, which is also a hydrophobic molecule. This might be the reason free catechins have essentially no effect on elimination of micellar cholesterol. Because Kajiya et al. (*19*) showed that the affinity for lipid bilayers and stereochemical structures were different among gallate esters of catechins, these differences might explain the differential effect on micellar solubility of cholesterol among **3**, **4**, **7,** and **8**.

Yang and Koo (*10*) reported that Lung Chen tea, a Chinese green tea, the major ingredient of which was **4**, increased fecal excretion of both cholesterol and bile acids in rats. Chan et al. (*11*) also reported that jasmine green tea catechins, mainly composed of **2** and **4**, increased fecal output of neutral and acidic steroids in hamsters. There are two possibilities for the enhanced excretion of bile acids in feces. One is that catechins inhibit reabsorption of bile salts in the ileum, probably by sequestering bile salts in the lumen. In the present study, catechins added to bile salt micelles did not affect the concentration of bile acids in the micellar solution. Therefore, it seems that catechins do not associate with bile salts. However, there is a possibility that the formation of a water-soluble complex of catechins with bile salts disrupted bile salt micelles, and consequently, cholesterol was precipitated. The second possibility is that catechins enhance the conversion of cholesterol to bile acids in the liver. Yang and Koo (*10*) observed that Chinese green tea tended to increase, but not significantly, the activity of cholesterol 7α-hydroxylase, the rate-limiting enzyme of bile acid synthesis, in rat liver. Therefore, more detailed studies are necessary in this regard.

Investigations on the safety of heat-epimerized catechins are scarce. In two studies (*18*, *20*), when tea drinks containing green tea catechins and heat-epimerized catechins (450−900 mg/day as total catechins) were given to humans for 8−20 weeks, no abnormality was observed on biochemical blood parameters. Because no information on the absorption and metabolism of heat-epimerized catechins is available, more studies on their safety, absorption, and metabolism should be done.

It has been known that green tea catechins have antioxidative activities on Cu^{2+}-mediated oxidation of low-density lipoproteins in vitro (*21*) and in human study (*22*, *23*). Unno et al. reported that $^{\bullet}O_2^-$ scavenging potentials of **5**−**8** in vitro were comparable to those of **1**−**4**, respectively (24), suggesting that heat-epimerized catechins have the same antioxidative activities on low-density lipoprotein oxidation as green tea catechins. Low-density lipoprotein oxidation has been shown to participate in the formation of atherosclerotic plaque (*25*). The present study showed a possibility that heat-epimerized catechins increased during retort pasteurization may be more effective to lower plasma cholesterol concentration than the parent tea catechins. It has also been established that plasma cholesterol concentration is an independent risk factor for atherosclerosis (*26*). Therefore, although both green tea catechins and heat-epimerized catechins may be effective to prevent atherosclerosis by plasma-cholesterol-lowering and antioxidative activities, our results suggest that heat-epimerized catechins are more effective than green tea catechins. Research on the prevention of atherosclerosis by heat-epimerized catechins is now in progress.

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