Note

Cholesterol-Lowering Effect of Black Tea Polyphenols, Theaflavins, Theasinensin A and Thearubigins, in Rats Fed High Fat Diet

Yuji MIYATA1,5, Takashi TANAKA2, Kei TAMAYA3, Toshiro MATSU4, Shizuka TAMARU1 and Kazunari TANAKA*1

1 Department of Nutrition, University of Nagasaki, 1-1-1 Manabino, Nagayo-cho, Nishisonogi-gun, Nagasaki 851-2195, Japan
2 Graduate School of Biochemical Science, Nagasaki University, 1-14 Bukyo-machi, Nagasaki 852-8521, Japan
3 Industrial Technology Center of Nagasaki, 2-1303-8 Ikeda, Ohmura, Nagasaki 856-0026, Japan
4 Faculty of Agriculture, Graduate School of Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
5 Agriculture and Forestry Technical Development Center, Nagasaki Prefectural Government, Higashisonogi, Nagasaki 859-3801, Japan

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Rats were fed a high fat diet containing 0.2% black tea polyphenols, theaflavins, theasinensin A or thearubigins for 4 weeks. The hepatic cholesterol concentration was significantly lower in rats fed the theaflavins, theasinensin A or thearubigins diet than in those fed the control diet without black tea polyphenols. Theasinensin A and thearubigins significantly accelerated fecal neutral and acidic steroid excretion, respectively. These results suggest that theasinensin A and thearubigins might induce the hepatic cholesterol-lowering activity through the promotion of fecal steroid excretion.

Keywords: theaflavins, theasinensin A, thearubigins, black tea polyphenols, fecal cholesterol excretion

Introduction

Green tea is commonly consumed in Japan, and is believed to have effects beneficial for human health. In the manufacture of green tea, harvested fresh leaves are immediately steamed to inactive the enzymes, especially polyphenol oxidase. Consequently, a relatively large amount of catechins remain in green tea leaves, because the oxidation of catechins is inhibited. It has been reported that tea catechins have antiobesity, anti-diabetic, hypocholesterolemic, hypotriacylglycerolemic, antioxidative and anticarcinogenic effects (Fallon et al., 2008; Ikeda et al., 2005; Islam and Choi, 2007; Ito et al., 2008; Kobayashi et al., 2005; Suzuki et al., 2005; Yoshono et al., 1994; Zhang et al., 2002). Black tea is one of the most popular beverages in the world. Black tea is manufactured by fermenting green tea leaves. Matsumoto et al. (1998) have found that black tea exerts hypocholesterolemic and hypotriacylglycerolemic effects in cholesterol-fed rats. Keemun black tea extract has been observed to reduce body weight in rats (Du et al., 2005). Thus, black tea appears to improve lipid metabolism and body fat deposition. In the manufacturing process of black tea, monomeric catechins undergo oxidative polymerization by the action of polyphenol oxidase contained in green tea leaves, leading to the formation of black tea polyphenols such as theaflavins (TFs), theasinensins (TSs), and thearubigins (TRs). TFs are categorized into the following forms: theaflavin, theaflavin-3-O-gallate, theaflavin-3′-O-gallate, theaflavin-3′,3′-di-O-gallate, and they characteristically occur as dimers of (-)-epigallocatechin (EGC) and (-)-epicatechin (EC) or their galloyl esters. TSs are a dimer of EGC and its galloyl ester, and theasinensin A (TSA) is a major component of TSs. TRs are polymer-like polyphenols, and are the main components of black tea polyphenols; however, they have not yet been chemically characterized because of their complexity. TF-enriched green tea consumption has been shown to reduce serum cholesterol concentration in hypercholesterolemic adults (Maron et al., 2003). TFs have been indicated to play a role in decreased intestinal cholesterol absorption via inhibition of micelle formation in vitro (Vermeir et al., 2008). Thus, although TFs have been reported to have hypocholesterolemic activities, there is little information on the cholesterol metabolism of TFs, TSA and TRs in serum and liver cholesterol concentra-
methylsilyl derivatives with 5α-cholestan-3-ol and methylation with diazomethane and acetylation with 23-nordeoxycholic acid, respectively. Data are expressed as mean ± SE, and were analyzed by ANOVA followed by the Tukey Kramer test to detect significant differences between group means.

Results and Discussion

Body weight, food intake and cholesterol concentrations of serum and liver are shown in Table 1. No significant differences were observed in final body weight and food intake among the four groups. Serum cholesterol concentration was comparable among all groups. The concentration of liver cholesterol was markedly reduced in the TFs, TSA and TRs groups when compared to the control group.

The dry fecal weight and steroid excretion are presented in Table 2. There were no significant differences in fecal weight among the four groups. Neutral steroid (cholesterol and coprostanol) excretion was significantly accelerated in rats fed the TSA diet when compared to rats fed the control diet. Acidic steroid excretion was significantly higher in the TR group than in the control group. Consequently, TSA and TRs induced the increase of total excretion of neutral and acidic steroids.

Vermeer et al. (2008) have shown that TFs reduced the incorporation of cholesterol into mixed micelles in vitro. Recently, Ikeda et al. (2010) have reported that theaflavin-monogallates decreased micellar solubility of cholesterol, resulting in the suppression of cholesterol absorption in the small intestine. In the present study, neutral steroid excretion was slightly, but not significantly, accelerated in rats fed TFs. It is possible that TFs induced the enhancement of fecal neutral steroid excretion through inhibiting the incorporation of cholesterol into mixed micelles, but the effect does not necessarily appear to be intense.

Feeding of the TSA diet significantly increased fecal neutral steroid excretion when compared to feeding the control diet without black tea polyphenols. Although there is little

### Materials and Methods

TFs, TSA and TRs were derived from fermented tea obtained by tea-rolling processing of green tea leaves and loquat leaves (Miyata et al., 2009), and isolated and refined by the method of Tanaka et al. (2009). Male Sprague-Dawley (SD) rats weighing 90–100 g (4-weeks-old) were obtained from SLC (Hamamatsu, Japan), and maintained on a commercial chow (type CE-2, Clea Japan, Tokyo, Japan). After acclimatization for 7 days, the rats, weighing about 140 g, were divided into four groups of equal body weight. The control diet was prepared according to the formula recommended by the American Institute of Nutrition (1977), and contained (in weight %): casein, 20; corn starch, 15; lard, 24; corn oil, 1; cellulose, 5; mineral mixture (AIN-76), 3.5; vitamin mixture (AIN-76), 1; dl-methionine, 0.3; choline bitartrate, 0.2; and sucrose to 100. TFs, TSA and TRs were added at the level of 0.2% to the control diet at the expense of sucrose. The rats had free access to the diets and deionized water for 4 weeks. The food consumption and body weight of each animal were recorded every day. Feces were collected for 2 days before sacrifice. After the rats were fasted for 6 h, the animals were decapitated and the blood collected, and the liver was immediately excised and weighed. All animal studies were carried out according to the guidelines for animal experiments at University of Nagasaki (Nagasaki, Japan), and under Law No. 105 and Notification No. 6 of the Government of Japan. Serum cholesterol was assayed enzymatically using a commercial kit (Cholesterol E-Test, Wako Pure Chemical Industries Ltd., Osaka, Japan). Liver lipids were extracted by the method of Folch et al. (1951). The concentrations of liver cholesterol were measured using a commercial kit. The amount of neutral and acidic steroids excreted into feces was measured by gas-liquid chromatography using a Supelcowax (SPB™-1) column (30 m × 0.25 mm × 0.25 μm film thickness) after transformation to tri-

### Table 1. Effects of dietary theaflavins, theasinensin A and thearubigins on body weight, food intake and serum and liver cholesterol concentrations in rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TFs</th>
<th>TSA</th>
<th>TRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>141 ± 4</td>
<td>143 ± 3</td>
<td>142 ± 3</td>
<td>143 ± 3</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>333 ± 11</td>
<td>336 ± 7</td>
<td>352 ± 10</td>
<td>345 ± 7</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>16.0 ± 0.7</td>
<td>16.2 ± 0.7</td>
<td>16.7 ± 0.6</td>
<td>16.4 ± 0.4</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dL)</td>
<td>81 ± 10</td>
<td>67 ± 5</td>
<td>68 ± 7</td>
<td>72 ± 5</td>
</tr>
<tr>
<td>Liver cholesterol (mg/g)</td>
<td>9.21 ± 0.61</td>
<td>4.21 ± 0.21</td>
<td>3.20 ± 0.24</td>
<td>4.47 ± 0.15</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SE for 6–7 rats.

TFs, theaflavins; TSA, theasinensin A; TRs, thearubigins

Rats were given a diet containing 0.2% theaflavins, theasinensin A and thearubigins for 4 weeks.

* Different letters show significant difference at p < 0.05.
information on the effects of TSA on micellar solubility of cholesterol, the increment of neutral steroid excretion into feces on feeding TSA may be exerted by diminishing micellar solubility of cholesterol.

Catechins reportedly did not promote the excretion of acidic steroid into feces (Kobayashi et al., 2005). Ikeda et al. (2010) observed that catechins and TFs added to bile salt micelles did not affect the concentration of bile acid in micellar solutions, and it was thought that catechins and TFs do not influence bile acid reabsorption. Since TRs accelerated acidic steroid excretion into feces, it may have a bile acid binding capacity and inhibit the reabsorption of bile acid in the small intestine. It has been reported that the hydrophobic interaction with bile acids inhibited absorption of bile acids (Iwami et al., 1986). However, it is unknown whether TRs, which are hydrophilic high molecular polyphenols, directly interact with bile acids in mixed micelles. Therefore, more detailed studies are necessary in this regard.

TSA significantly reduced hepatic cholesterol level. This reduction is considered to be, in part, exerted by the increment of fecal steroid excretion. High excretion of neutral steroid into feces diminishes the amount of cholesterol returned to the liver through the entero-hepatic circulation system, resulting in the reduction of liver cholesterol concentration. On the other hand, Abe et al. (2000) have found that TSA potently inhibited rat squalene epoxidase, a rate-limiting enzyme of cholesterol synthesis. There is a possibility that low cholesterol level in rats fed TSA was exerted through the inhibition of cholesterol biogenesis in liver.

TRs also exerted the reduction of hepatic cholesterol level. The increment of fecal bile acid excretion decreases the extent of cholesterol solubility in bile salt micelles and hence, reduces the opportunity of intestinal absorption of cholesterol and decreases cholesterol concentration in liver. Another possible mechanism proposed for the reduction of hepatic cholesterol concentration by feeding TRs is the stimulation of hepatic formation of bile acid from cholesterol to compensate the acceleration of fecal acidic steroid excretion.

TFs may induce liver cholesterol-lowering activity through the increment of neutral and acidic steroid excretion, but because the amount of fecal steroid excretion by feeding TFs is somewhat less than those by feeding TSA and TRs, other mechanisms for the reduction of hepatic cholesterol level may be involved.

Thus, the effects of TFs, TSA and TRs on cholesterol metabolism seem to be respectively different, and the cause may be due to the structural differences of black tea polyphenols. More studies on the function of individual black tea polyphenols should be performed in the future.

The present study, for the first time, indicated that TSA and TRs as well as TFs lower hepatic cholesterol concentration via increasing fecal steroid excretion.

<table>
<thead>
<tr>
<th>Table 2. Effects of dietary theaflavins, theasinensin A and thearubigins on neutral and acidic steroid excretion in rats.</th>
<th>Control</th>
<th>TFs</th>
<th>TSA</th>
<th>TRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry feces weight (g/d)</td>
<td>1.69 ± 0.10</td>
<td>2.46 ± 0.46</td>
<td>1.79 ± 0.14</td>
<td>1.94 ± 0.12</td>
</tr>
<tr>
<td>Neutral steroids (mg/d)</td>
<td>3.87 ± 0.27ab</td>
<td>5.02 ± 0.52ab</td>
<td>5.11 ± 0.33b</td>
<td>4.35 ± 0.32ab</td>
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<tr>
<td>Acidic steroids (mg/d)</td>
<td>3.15 ± 0.23ab</td>
<td>4.04 ± 0.31ab</td>
<td>4.26 ± 0.42ab</td>
<td>5.08 ± 0.42ab</td>
</tr>
<tr>
<td>Total steroids (mg/d)</td>
<td>7.03 ± 0.42ab</td>
<td>9.06 ± 0.52ab</td>
<td>9.37 ± 0.43b</td>
<td>9.43 ± 0.72ab</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SE for 6 – 7 rats.
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References


