Effect of Dietary Green Tea Catechin Preparation on Oxidative Stress Parameters in Large Intestinal Mucosa of Rats

Masako YAMAMOTO,1 Sayuri MIYAMOTO,2,* Jae-Hak MOON,2,**
Kaeko MUROTA,2 Yukihiko HARA,3 and Junji TERAO2,†

1Department of Life Science, Tokushima Bunri Junior College, Tokushima 770-8514, Japan
2Department of Food Science, Graduate School of Nutrition and Biosciences, The University of Tokushima, Tokushima 770-8503, Japan
3Mitsui Norin Company, Ltd., Nishishinjuku, Shinjuku-ku, Tokyo 160-8381, Japan

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** Present address: College of Agricultural and Life Sciences, Chonnam National University, 300 Yongbong-Dong, Buk-gu, Gwangju 500-757, Korea

Note

Intake of green tea catechin (GTC) for 4 weeks was found to elevate vitamin E level in the mucosa of the rat large intestine. Iron-induced lipid peroxidation of the mucosal homogenate was suppressed by intake of GTC in rats fed monounsaturated fatty acid (MUFA), indicating that the protective effect of dietary GTC on mucosal oxidative stress is enhanced by combination with a MUFA-rich diet.

Key words: tea catechin; lipid peroxidation; intestinal mucosa; oxidative stress; antioxidant

Green tea catechins (GTCs) act as antioxidants by scavenging reactive oxygen species (ROS) and chelating metal ions which promote the generation of ROS.1,3 The potential health effects of tea drinking might be at least partly attributable to their antioxidant activity,2 since ROS are known to participate in degenerative diseases. However, attention should be paid to the fact that GTCs or their isolated catechin can act as prooxidants by generating H₂O₂ spontaneously.3,4 Dietary GTCs are somewhat absorbed from the intestinal tract and circulate in the blood stream in their intact form and/or as metabolites.5 Nevertheless, a large portion of GTCs is transferred into the colon, where the resident microflora degrade them to smaller phenolic acids, some of which are absorbed.6 It has been suggested that the gut lumen is an alternative site for the health impact of unabsorbed polyphenols.7 Thus GTCs might exert a direct effect within the gastrointestinal tract, since they can be found in high concentrations in the digestive tract.8 Matsumoto et al.9 found that dietary GTCs inhibit colonic mucosal lipid peroxidation in 1,2-dimethylhydrazine-induced rat colonic carcinogenesis, but little is known as to how dietary GTCs affect oxidative stress in colonic mucosa. Hence we tried to evaluate the effect of dietary GTCs on oxidative stress parameters in large intestinal mucosa, in particular, in those related to iron-induced lipid peroxidation, using rats fed two different types of diets.

The treatment of animals conformed to the “Guidelines for the Care and Use of Experimental Animals, Tokushima Bunri University”. Wistar male rats (4 weeks old) obtained from Japan SLC (Hamamatsu, Japan) were randomly divided into four groups. During a period of 4 weeks, five rats in each group were fed one of the following 4 experimental diets: (1) a PUFA-rich corn oil diet (PUFA), (2) a PUFA-rich corn oil diet containing a 3% GTC preparation (PUFA + GTC preparation), (3) a MUFA-rich safflower oil diet (MUFA), and (4) a MUFA-rich safflower oil diet containing a 3% GTC preparation (MUFA + GTC preparation). We used high-oleic acid type safflower oil which is rich in oleic acid (Nissin Oil, Tokyo). The fatty acid composition of each oil was determined by gas-liquid chromatography (GLC) analysis as follows: in corn oil (PUFA), 16:0 (11.5 ± 0.6%), 18:0 (1.7 ± 0.2%), 18:1 (27.8 ± 0.3%), 18:2 (58.9 ± 0.2%); in safflower oil (MUFA), 16:0 (4.8 ± 0.2%), 18:0 (2.0 ± 0.1%), 18:1 (77.3 ± 0.03%), 18:2 (15.9 ± 0.1%) respectively. GTC preparation was supplied as Polyphenon-70S® by Mitsui Norin (Tokyo). The composition of each catechin in this preparation was as follows: (-)-epigallocatechin gallate (EC-g) 31.7, (-)-epigallocatechin (EGC) 15.7, (-)-epicatechin gallate (EC-g) 10.0, (-)-epicatechin (EC) 8.5, and others 34.1 (wt%). The vitamin E level was equalized in all diets by supplementing RRR-α-tocopherol (Acros Organic Co., Morris Plains, NJ) to corn oil for adjusting...
buffer (0.1 M, pH 7.4) containing 0.135 M KCl. The
and then homogenized with 6 volume of Tris–HCl
scraped with a glass plate and washed with 0.9% NaCl
intraperitoneal administration) after overnight fasting.

cycle). At the end of the study, rats were anesthetized
posthoc multiple comparison using Stat-View software
Statistical analysis was evaluated by one-factor analysis
Results are presented as the

mucosa were measured as described previously.10) The
vitamin E content and fatty acid composition of the
mucoa homogenate, with Fe(NO$_3$)$_3$, and ascorbic acid
uptake was estimated by thiobarbituric acid reactive
resistance of large intestinal mucosa to lipid peroxida-

<table>
<thead>
<tr>
<th>Ingredients (wt %)</th>
<th>PUFA group</th>
<th>MUFA group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn oil</td>
<td>Corn oil + GTCs</td>
</tr>
<tr>
<td></td>
<td>MUAFA</td>
<td>MUAFA + GTCs</td>
</tr>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Starch</td>
<td>67</td>
<td>64</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mineral mixture*</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin mixture*</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fiber</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>GTCs</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>$\alpha$-Tocopherol**</td>
<td>1.58</td>
<td>1.58</td>
</tr>
</tbody>
</table>

*Mineral mixture and vitamin mixture were obtained from Oriental Yeast (Tokyo).
**($\mu$g/100 g) $\alpha$-Tocopherol was added to the oil to adjust the vitamin E level in the diet for each group.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>PUFA</th>
<th>PUFA + GTC Preparation</th>
<th>MUFA</th>
<th>MUFA + GTC Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>23.9 ± 2.7</td>
<td>23.4 ± 1.7</td>
<td>25.1 ± 1.7</td>
<td>25.1 ± 1.5</td>
</tr>
<tr>
<td>18:0</td>
<td>11.6 ± 0.7</td>
<td>12.7 ± 0.8</td>
<td>9.5 ± 1.3</td>
<td>11.5 ± 1.0</td>
</tr>
<tr>
<td>18:1</td>
<td>29.4 ± 2.1</td>
<td>26.7 ± 1.5</td>
<td>42.8 ± 3.3</td>
<td>38.6 ± 2.6</td>
</tr>
<tr>
<td>18:2</td>
<td>14.6 ± 0.3</td>
<td>16.3 ± 1.3</td>
<td>7.1 ± 0.7</td>
<td>9.3 ± 0.6</td>
</tr>
<tr>
<td>20:4</td>
<td>19.6 ± 3.4</td>
<td>18.9 ± 1.2</td>
<td>14.5 ± 2.8</td>
<td>16.8 ± 2.5</td>
</tr>
<tr>
<td>22:6</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>1.0 ± 0.3</td>
<td>1.2 ± 0.3</td>
</tr>
</tbody>
</table>

Peroxidizability index (PI)**
PUFA content (mg/g tissue)**
Vitamin E content (mg/100 g tissue)

Table 2. Fatty Acid Compositions and Vitamin E Levels of Large Intestinal Mucosa at the End of the Study

Table 1 shows the composition of each diet. The animals were housed under standard laboratory conditions (23 °C, 60% humidity, and 12 h light 12 h dark cycle). At the end of the study, rats were anesthetized with sodium pentobarbital (0.1 ml/100 g body weight intraperitoneal administration) after overnight fasting. The large intestine was removed and flushed with cold saline to remove intestinal contents. The mucosa was scraped with a glass plate and washed with 0.9% NaCl and then homogenized with 6 volume of Tris–HCl buffer (0.1 M, pH 7.4) containing 0.135 M KCl. The vitamin E content and fatty acid composition of the mucosa were measured as described previously. The resistance of large intestinal mucosa to lipid peroxidation was estimated by thiobarbituric acid reactive substances (TBARS), with and without incubating mucosa homogenate, with Fe(NO$_3$)$_3$, and ascorbic acid (100 μM and 1 mM, respectively) for 4 h at 37 °C. Results are presented as the means ± SD of five rats. Statistical analysis was evaluated by one-factor analysis of variance (ANOVA) followed by Bonferroni/Dunn post hoc multiple comparison using Stat-View software (ver. 5, SAS institute). A difference of $p < 0.05$ was considered significant.

Table 2 shows the fatty acid composition and the PUFA content of the mucosa of each group at the end of the study. In the MUFA and MUFA + GTC preparation groups, there was an increase of 18:1 (oleic acid) and a decrease of 18:2 (linoleic acid) as compared with the PUFA and PUFA + GTC preparation groups. The PUFA contents in the MUFA and MUFA + GTC preparation groups were also lower than those in the PUFA and PUFA + GTC preparation groups. The decrease in PUFA content resulted in lower peroxidizability indexes (PI) for the MUFA and MUFA + GTC preparation groups. It is important to note that supplementation of the diet with GTCs did not alter the fatty acid composition or PI. Table 2 also shows the vitamin E ($\alpha$-tocopherol) contents in the large intestinal mucosa of the four groups at the end of the study. In both types of diet, supplementation with GTC preparation (MUFA + GTC preparation and PUFA + GTC preparation group) led to a significant increase in the level of vitamin E.

In general, lipid peroxidation proceeds via the
initiation process and subsequent free radical chain reaction, producing lipid hydroperoxides and their decomposition products. Radical chain reaction occurs in the PUFA moiety of membranous lipids, where lipophilic antioxidants, such as vitamin E, can interrupt the chain reaction. Therefore, oxidative stress originated from lipid peroxidation is affected by the amounts of PUFA to be oxidized and those of lipophilic antioxidants. In our experiments, the MUFA/PUFA contents in the diet, but not GTCs, influenced the fatty acid composition and PUFA contents of large intestinal mucosal lipids. The PI of the mucosal lipids in the PUFA-rich diet was therefore higher than that from the MUFA-rich diet. Thus, it can be predicted that the large intestinal mucosa of rats fed a PUFA-rich diet is more susceptible to radical chain lipid peroxidation. On the other hand, our result also indicates that dietary GTCs increased the vitamin E level in the mucosa regardless of the MUFA/PUFA contents of the diet. We have suggested that (-)-EC and (-)-EGC are localized near the surface of phospholipid membranes for scavenging aqueous oxygen radicals and thereby preventing the consumption of lipophilic vitamin E in a liposomal model membrane system.13) Hence interaction of GTCs with the membranes, as indicated by Kumazawa et al.,14) facilitate protection of vitamin E from ROS attack on the luminal side.

Table 3 shows the result of TBARS assay for the large intestinal mucosa homogenate of each group. TBARS contents increased dramatically on incubation with iron, indicating that iron efficiently induced lipid peroxidation in the mucosa homogenate in vitro. Comparing all groups, only the mucosa of the MUFA-rich diet was higher than that from the MUFA/PUFA preparation group, which might be located on the surface of the large intestinal bacteria. But a considerable portion of GTCs might be located on the surface of the large intestinal mucosa, where they can exert a chelating effect on iron coming from the efflux of diet. Alternatively, several decomposition products formed by microflora might act as antioxidants in the intestine.

In conclusion, supplementation with GTC preparation enhances the resistance of the large intestinal mucosa against iron-induced lipid peroxidation efficiently when it is combined with a MUFA-rich diet. GTC preparation also enhanced the vitamin E concentration in the large intestinal mucosa. Thus, intake of GTC appears to be helpful in the protection of mucosal lipids of the large intestine from oxidative stress. In addition, it should be noted that GTC preparation did not act as a prooxidant in the intestinal mucosa, as indicated by the vitamin E level and TBARS content without incubation (Tables 2 and 3), although an excess amount of GTCs was administered to the rats in this study. Further investigation will be required to assess the influence of dietary fats and oils on the action of GTCs in the intestinal lumen.

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