Protective effect of tea catechins on lipid peroxidation in intestinal mucosa.

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Summary
Green tea catechins are effective antioxidants by scavenging reactive oxygen species (ROS) and/or chelating metal ions. It is believed that catechins are absorbed and accumulated in the body as both intact form and conjugated metabolites. Some metabolites seem to be secreted into intestinal lumen through enterohepatic circulation. Thus, an appreciable amount of catechins remains in the lumen as metabolites or their hydrolyzed form. However, little is known about their role in the antioxidant defense of digestive tract. We have found in rat that large intestinal mucosa is more susceptible to iron ion-induced lipid peroxidation as compared to gastric and small intestinal mucosa. Thus, we examined the protective effect of tea catechins on iron ion-induced lipid peroxidation of large intestinal mucosa using rats fed linoleic acid-rich corn oil or high oleic acid-safflower oil and tea catechins. Peroxidizability of mucosa homogenate was lower in the rats receiving safflower oil and catechins. Mucosa vitamin E level was higher in the rats receiving catechins, as compared with those receiving no catechins. These results indicate that supplementation of green tea catechins with high oleic acid-rich oil provides protection against metal ion-induced oxidative stress in the large intestinal mucosa.

Keywords
Tea catechins, Lipid peroxidation, Vitamin E, Large intestine, Intestinal mucosa

Introduction
Tea catechins have attracted much attention in relation to their potential beneficial effect. In particular, their antioxidant property is frequently referred from the viewpoint of the prevention for degenerative diseases such as cancer and cardiovascular disease. Tea catechins comprise of four major compounds, epigallocatechin gallate (EGC-g), epicatechin gallate (EC-g), epigallocatechin (EGC) and epicatechin (EC). They are all able to act as antioxidants by scavenging reactive oxygen species (ROS) and chelating metal ions which promote the generation of ROS. Fig.1 shows the number of molecules of a stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), scavenged by one molecule of each tea catechin component¹. It is clear that DPPH radical-scavenging capacity of each catechin is higher than that of vitamin E or vitamin C. EGC-g possesses the highest capacity among them. In addition, EGC-g seems to possess a high chelating activity, because EGC-g gave a strong inhibition on deoxyribose assay² for measuring chelating activity (Fig.2). EGC-g is the predominant among for catechins in tea indicating that antioxidant effect of tea catechins can be expected in digestive tract by their intake of tea catechins.

Tea catechins have shown inhibitory effect against colon carcinogenesis in experimental animal model studies³. This effect was suggested to be associated with the prevention of mucosa oxidative damage. In the colon, ROS can be generated by the intestinal flora. Furthermore, dietary iron may act as catalyst of oxidative reactions⁴. Thus, we tried to evaluate the effect of tea catechins on the susceptibility of large intestinal mucosa against iron-ion induced oxidation in rats fed different types of fats. We used high oleic acid-safflower oil (HO-safflower oil) and compared its effect to the conventional corn oil (rich in linoleic acid).
Materials and Methods
Wistar male rats were divided into 4 groups and treated with the following diets for 4 weeks: 5% corn oil, 5% corn oil and 3% tea catechins, 5% OH-safflower oil; 5% OH-safflower oil and 3% tea catechins. Large intestinal mucosa was scrapped and washed in cold saline. The mucosa obtained was homogenized with 6 vol of Tris-HCl buffer (0.1 ml, pH 7.4 containing 0.135 M KCl). Peroxidizability of the mucosa homogenate was measured by incubating it with Fe(NO$_3$)$_3$ and ascorbic acid (100 $\mu$M and 1mM, respectively). Aliquots were withdrawn and the oxidation level was determined by TBA-fluorometric assay. The fatty acid composition of mucosa homogenate was determined by gas-liquid chromatographic analysis (GLC) after lipid extraction and transmethylation. The tocopherol content of mucosa homogenate was measured by a sensitive fluorometric-HPLC method according to the method of Hatam and Kayden. EGC-g in the homogenate was measured by HPLC technique using electrochemical detection.

Results and Discussion
Major fatty acid components in corn oil and OH-safflower oil were linoleic acid (18:2, 58.9 %) and oleic acid (18:1, 77.3 %), respectively. Table I shows the contents of 18:1, 18:2, other unsaturated fatty acids and $\alpha$-tocopherol in large intestinal mucosa of four groups after the trial. Two OH-safflower oil groups decreased 18:2 content in concomitant with the increase of 18:1. This change apparently reflects the respective fatty acid composition of each oil in the diet. In both types of the diet, supplementation with catechins increased the level of $\alpha$-tocopherol in the large intestinal mucosa. Table I also shows the TBARS content in the mucosa of each group after the incubation with ferrous ion. The result that only OH-safflower oil and catechin group gave the significantly lower TBARS level indicates that dietary catechins exert protective effect against iron ion-catalyzed lipid peroxidation of large intestinal mucosa in the group of rats receiving OH-safflower oil diet. Intake of high-oleic acid diet is likely to decrease the peroxidizability of intestinal mucosa against lipid peroxidation by elevating oxidation-resistant monounsaturated fatty acids in the lipid portion. Combination of high oleic acid-oil and tea catechin in the diet actually helps in the protection against ROS-induced oxidative damage in intestinal mucosa. In both tea catechin supplemented groups, EGC-g was detected in the large intestinal mucosa homogenate at the
Table 1 Parameters for peroxidizability of large intestinal mucosa from rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Corn oil group</th>
<th>SO-Safflower oil group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Com oil</td>
<td>Com oil + catechins</td>
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<tr>
<td>Fatty acid (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:1</td>
<td>29.4±2.1</td>
<td>26.7±1.5</td>
</tr>
<tr>
<td>18:2</td>
<td>14.6±0.3</td>
<td>16.3±1.3</td>
</tr>
<tr>
<td>Lipid (mg/ml)</td>
<td>0.54±0.07</td>
<td>0.47±0.05</td>
</tr>
<tr>
<td>PUFA (mg/ml)</td>
<td>0.19±0.02</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>( \alpha )-tocopherol ((\mu\text{mol/g tissue}))</td>
<td>3.81±0.17</td>
<td>9.97±0.17*</td>
</tr>
<tr>
<td>TBARS (nmol/g tissue after incubation)</td>
<td>249±104</td>
<td>238±86</td>
</tr>
</tbody>
</table>

Values are means ± SD. Significance: *p<0.05 and **p<0.01

Fig. 3 Proposed mechanism of tea catechin action on \( \alpha \)-tocopherol sparing in intestinal mucosa

content of 1.61±0.24 nmol/g mucosa and 2.02±1.40 nmol/g mucosa, respectively. It is known that tea catechins in the diet mostly reached to large intestine without absorption into the body and decomposed into ring-scission products by the action of intestinal bacteria. However, our results suggest that considerable part of tea catechins is able to bind or adhere to the surface of large intestinal mucosa and thereby act as effective antioxidants against the attack of ROS from the tract. On the other hand, hydrophobic \( \alpha \)-tocopherol is believed to be located in the interior of the mucosa matrix. Thus, tea catechins seems to spare \( \alpha \)-tocopherol by inhibiting the generation of ROS due to Fenton reaction and/or scavenging chain-carrying lipid peroxy radical generated in mucosa surface (Fig. 3). In conclusion, supplementation of tea catechins with oleic acid-rich oil is helpful to elevate the resistance against metal ion-induced oxidative damage in large intestinal mucosa. Diet including tea catechins and high oleic acid-oil may provide protection against ROS attack in intestinal mucosa.
References