Absorption of Tea Catechins into Rat Portal Vein

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Following the oral administration of tea catechins, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate and (−)-epigallocatechin gallate, respectively, to rats, the presence of these catechins in the portal blood was examined. It was confirmed by HPLC and mass spectrometry analysis that each of the administered catechins was present in the blood. These results clearly indicate that four predominant catechins in fresh tea leaves are absorbed, at least in part, into the rat portal vein.

Key words tea catechin; absorption; portal blood; rat

Tea catechins are the major components of fresh tea leaves, as well as the soluble matter in green tea. The principal catechins present in green tea are (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECg), (−)-epigallocatechin gallate (EGCg). These catechins have been proved to have a variety of physiological functions.1) It is noteworthy that the catechins have in vitro and in vivo antioxidative activity which may be closely related to their preventive effects on various diseases2) including liver injury, arteriosclerosis and inflammation caused by lipid peroxidation and excessive free radical production. In addition, tea catechins and EGCg have been reported to function as cancer chemopreventive agents in many different animal models.3) However, limited information is available on the metabolism of the compounds, even on their absorption in the body. Recently, we reported that EGCg is absorbed into portal blood via the intestinal tract in the rat.4) In the present study, we describe the absorption of tea catechins EC, EGC, and ECg, in addition to EGCg.

MATERIALS AND METHODS

Materials EC, EGC, ECg and EGCg were prepared from green tea by our method previously reported.5) The chemical structure of each tea catechin is illustrated in Fig. 1. Tannase (from Aspergillus oryzae) was obtained from Sankyoo Co., Ltd., Tokyo. All other chemicals were available products of analytical grade.

Animals and Diets Male Wistar rats (6 weeks of age) were fed a powder-type diet. The composition of the diet is shown in Table 1. The diet was prepared with highly purified compounds in order to minimize the content of flavonoids and other plant phenolics.

Animal Experiments Each of the tea catechins (100 mg) was dissolved in 2 ml of distilled water. The solution was administered orally to the rats after they were starved overnight. The rats were anesthetized with pentobarbital sodium 45 min after dosing, and blood was collected from

Table 1. Composition of Diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Corn starch</td>
<td>48.9</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
</tr>
<tr>
<td>Casein</td>
<td>25.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
</tr>
<tr>
<td>Salt mixture a)</td>
<td>4.0</td>
</tr>
<tr>
<td>Vitamin mixture b)</td>
<td>2.0</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.1</td>
</tr>
</tbody>
</table>

a) Salt mixture and vitamin mixture according to Harper were purchased from Oriental Kobo Kogyo Co.

Fig. 1. Chemical Structures of Tea Catechins

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the portal vein and heparinized. Plasma was separated from the blood by centrifugation at 3000 rpm for 10 min, and then extracted 3 times with 10 ml of ethyl acetate. The extract was evaporated to dryness and the residue was used as an analytical sample. In the case of the plasma obtained from EGCG administered rats, a compound represented by a peak whose retention time agreed with that of the standard EGCG was isolated by preparative HPLC and analyzed by FAB-MS, as described in our previous paper.4)

**Enzyme Hydrolysis** One-half of the residue was added to 1 ml of 0.1 M sodium phosphate buffer (pH 6.8) containing 1 unit of tannase. After being incubated for 3 h at 37 ℃, the reaction mixture was analyzed by HPLC.

**Analytical Methods** HPLC was performed in a JASCO liquid chromatograph apparatus with a JASCO 870 UV detector and with a model M996 photodiode array detector (Nihon Waters, Ltd.). The HPLC conditions were as follows: column, Capcell-pak C-18 AG; (4.6 x 250 mm, Shiseido Co., Ltd.); mobile phase, acetonitrile–ethyl acetate–0.05% phosphoric acid aqueous solution (12:2:86 by volume); flow rate, 1 ml/min; temperature, 40 ℃; detection, UV 280 nm or wavelength in the range of 230—450 nm. LC-MS (FRIT FAB ionization method) analysis was carried out using a JEOL JMS SX-102A mass spectrometer equipped with a Hitachi liquid chromatograph L-6000 apparatus. The LC conditions were the same as the above HPLC conditions except that acetonitrile–ethyl acetate–water (12:0.6:90 by volume) was used as a mobile phase and the analysis was carried out at room temperature.

**RESULTS**

Tea catechins, EC, EGC, ECG and EGCG, were given to the rats individually by oral administration. Each ethyl acetate fraction obtained from the portal blood was first examined by HPLC in order to determine whether these four individual catechins are absorbed into the body. Several peaks were detected in each of the ethyl acetate fractions, as shown in Fig. 2. On comparison of the retention time and UV spectrum of those peaks with those of standard catechins, in each fraction there was only one peak which coincided with the catechin administered. Further, the ethyl acetate fractions were treated with tannase, which is capable of hydrolyzing galloyl ester, and they were then analyzed by HPLC. The peak whose retention time corresponded to that of EC disappeared, and two new peaks were formed from the original peak. Judging from the comparison of the retention time and UV spectra with those of tea catechins and gallic acid, the new peaks were assumed to be gallic acid and EC. Similarly, after tannase treatment, two new peaks were formed from the peak whose retention time agreed with that of EGCG, and they were assumed to be gallic acid and EGC. Thus, the above results suggest that orally administered tea catechins into rats are absorbed to the portal blood.

In addition, the three ethyl acetate fractions derived from each sample of portal blood taken from the rats given EC, EGC, and ECG, respectively, were analyzed by LC-MS. In the case of EGCG administered to rats, the major compound was isolated and analyzed by FAB-MS, as described in "Materials and Methods." As shown in Fig. 3, the main compound in each of the fractions was identified as EC, EGC, ECg, and EGCG, respectively.

In this study, a first step in examining the fate of tea catechins in vivo, it was clearly demonstrated that tea catechins, EC, EGC, ECg and EGCG, administered orally to rats, are absorbed into portal blood via the intestinal tract.

**DISCUSSION**

We have shown in this study that tea catechins are absorbed, at least in part, into the portal blood in rats. This is the first report concerning the absorption of tea
catechins into the body. In the present study, it was necessary not only to identify the absorbed compounds but also to determine their contents. A preliminary attempt was made to determine the content of EC and EGCg in rat blood in vitro. The recovery rates of EC and EGCg from the blood are given in Table 2.

The extraction efficiency of both catechins tended to decrease as the incubation time increased. In particular, the recovery rates of EGCg were found to be lower than 50% of the compound added, even after 1 h incubation. These results implied that tea catechins may be adsorbed to plasma protein or blood cells and cannot be extracted
quantitatively by the method used in this study. Nevertheless, the present study will be of value in future investigations which seek to understand in more depth the physiological functions of tea catechins.

REFERENCES


