Note

Antioxidative Activity of (−)-Epigallocatechin-3-(3′-O-methyl)gallate Isolated from Fresh Tea Leaf and Preliminary Results on Its Biological Activity

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Antioxidative activity of (−)-epigallocatechin-3-(3′-O-methyl)gallate (catechin e) was examined. Catechin e showed a strong antioxidant activity. A preliminary test using rat cancer cells suggests that catechin e also has a strong cytotoxic activity. Among tested catechins, only catechin e has strong activity for both.

Key words: antioxidative activity; catechins; (−)-epigallocatechin-3-(3′-O-methyl)gallate

Catechins are major constituents of the tea leaf (approximately 15–25% of dry weight), and play a significant role in the biological and the pharmacological properties of tea, such as antioxidative,1) antiallergic,2) antimitogenic/anticarcinogenic,3,4) and antibacterial activities.5) (−)-Epigallocatechin-3-(3′-O-methyl)gallate (catechin e in Fig. 1) was isolated from the tea leaf, Benihomare, in 1982,6) and it was found that its content in tea leaf was very small (0.07–0.52% dry weight). Sano et al.7) reported that catechin e had a strong antiallergic activity.7) The other biological and the pharmacological properties of this compound, however, has never been reported because it is quite difficult to obtain catechin e due to its very small content in tea leaf.

In this paper, we will report the antioxidative activity of (−)-epigallocatechin-3-(3′-O-methyl)gallate (catechin e) and preliminary result of its effect on cultivated rat hepatoma cells. Catechins used in this study are shown in Fig. 1. Catechin e was isolated by the method of Saijo.8) Briefly, young tea leaves were homogenized in 80% acetone and then filtered. The filtrate was dried and dissolved in water. The aqeous phase was extracted with chloroform and then with methylisobutylketone. The crude catechin was obtained by the extraction to dryness of the methylisobutylketone phase with diethyl ether. The crude catechin was dissolved in water and then put on a Sephadex LH-20 column equilibrated with 40% acetone. The column was washed with 40% acetone, and then catechin e was eluted by a methanol-hexane-ethyl acetate-water mixture (100:30:25:5 v/v). Two fractions were obtained, and the later eluted fraction contained catechin e. The catechin e fraction was put on the Sephadex LH-20 column again, and then catechin e was eluted with methanol-chloroform-hexane (2:1:1 v/v) as the solvent.

The antioxidative activities of catechin e and other catechins were measured by a slightly modified β-carotene discoloration method of Tsushida et al.6) Briefly, the concentration of β-carotene and other chemicals in the substrate solution were equal to those in the method, and 0.1 ml of catechin (10 ppm) was added to 4.9 ml of the above substrate solution and incubated at 55°C. Absorbance at 470 nm of the resulting reaction mixture was measured every 5 min. Water was used as a control. The antioxidative activity of 3(2)-t-butyl-4-hydroxynisole (BHA) (10 ppm) was also tested. The typical course of change at 470 nm absorbance of sample, (−)-epicatechin-3-gallate (ECG), and BHA were illustrated in Fig. 2. A, B, and X in Fig. 2 showed the difference of absorbance between 5 min and 35 min of reaction of control, BHA, and ECG, respectively. We improved the expression of the antioxidative activity. The antioxidative activity in this study was expressed as an equivalent amount (mol) of BHA per 1 mol of sample by following equation 1 (1).

Antioxidative activity

\[
= \frac{(A - X)}{(A - B)} \times (M.W.)/180.25
\]

M.W. means the molecular weight of catechin, and 180.25 is the molecular weight of BHA. Tsushida et al. expressed the antioxidative activity as the equivalent concentration of catechin to 10 ppm of BHA.

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Antioxidative Activity of 3-O-Methyl Galloyl Catechin Derivative

**Fig. 2.** The Model Schemes of Change at 470 nm Absorbance of Control (■), (-)-Epicatechin-3-gallate (ECG) (●), and BHA (▲) in Measurement of Antioxidative Activity of Catechins. A, B, and X in figure show the difference of absorbance between 5 min and 35 min of reaction of control, BHA, and ECG, respectively.

**Table 1.** Antioxidative Activity of Catechins

<table>
<thead>
<tr>
<th>Catechin</th>
<th>Equivalent amount of BHA per 1 mol of each catechin* (mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>0.27</td>
</tr>
<tr>
<td>b</td>
<td>1.23</td>
</tr>
<tr>
<td>c</td>
<td>0.71</td>
</tr>
<tr>
<td>d</td>
<td>1.99</td>
</tr>
<tr>
<td>e</td>
<td>1.86</td>
</tr>
<tr>
<td>f</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* Values are the means of two experiments.

The expression in this study is considered to be easier to understand than their expression.

Table 1 shows the antioxidative activity of catechins (a-f). Catechin e is known to be present only in small amounts in tea leaf, and therefore has hardly been studied. Catechins e has a strong antioxidative activity equal to that of catechin d, which is known to have a strong antioxidative activity among catechins.

A voltammetric study was also done to examine the oxidation potential of catechins. Catechins d and e had lower oxidation potentials than those of other tested catechins (data not shown). There was, however, no difference in oxidation potential between catechins d and e. O-Methyl group in the galloyl group of catechin e is assumed not to affect the oxidation process of catechin e. To study the details...
of the oxidation process of catechin, energy or the distribution of electrons in catechin must be investigated. Therefore, the relationship between structure and antioxidative activity in catechin must be investigated in detail by using molecular orbital simulation. In a further study, we will report the results of molecular orbital simulation.

Generally, the antioxidative activity of catechin is said to correlate to its biological activity. The results in Table 1 also provide the expectation that catechin e also has strong biological activities apart from the antiallergic activity. The effect of catechins (a-f) on the cultivated rat cancer cells was also examined and a preliminary result was obtained.

A rat hepatoma cell line, H4-II-E-C3, was used in this study. Cultivation conditions and medium were almost the same as in the method of Kawase et al. In this study, a polystyrene plate for adhered culture was used instead of that for suspension culture. The final concentration of 0.02 mm of catechin was added to the culture medium. No catechin was added in a control run. After 5 days of cultivation of hepatoma cells, the cells on the plate were counted. The number of cells was significantly lower in the culture containing catechins than that in the control. In particular, the number of cells was very low (below 10% of that in the control) in the culture with catechins b and e. With the addition of other catechins including catechin d, the number of cells was in the range from 30 to 40% of that in control.

Catechin e, (−)-epigallocatechin-3-(3′-O-methyl) gallate, is suggested to have both a strong antioxidative activity and a strong cytotoxic activity to rat hepatoma cells in this study. The relationship between structure and antioxidative activity in catechins has not been clarified. The energy or distribution of electrons in catechin must be investigated to clarify such a relationship. The mechanism of the strong cytotoxic activity of catechin also is unclear. Especially, the role of the O-methyl group in the galloyl group of catechin e must be clarified in further study to apply catechin e to antitumor medicine.

References