Apoptosis-inducing Activity of Polyphenol Compounds Derived from Tea Catechins in Human Histiolytic Lymphoma U937 Cells

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Polyphenolic compounds derived from tea catechins were examined for apoptosis-inducing activity in human histiolytic lymphoma U937 cells. (−)-Epigallocatechin gallate, theasinensin D, compound OH-5, theaflavin, and theaflavin digallate induced apoptosis as evidenced by DNA ladder formation, its inhibition by a caspase inhibitor, and chromatin condensation. Theaflavins D was the most potent inducer and the data suggest the importance of the number and three dimensional localization of their phenolic groups in this activity. These apoptosis-inducible compounds may be useful as a cancer chemopreventive and chemotherapeutic agent.

Key words: apoptosis; catechin; epigallocatechin gallate; theaflavin; theasinensin D

Several chemotherapeutic compounds have been reported to induce apoptosis or programmed cell death, and apoptosis may be a primary mechanism of their anti-cancer activity. Recently, green tea catechins and black tea theaflavins have been shown to induce apoptosis in human lymphoblastic leukemia Molt 4B cells, promyelocytic leukemia HL-60 cells, stomach cancer KATO III cells, and other human cancer cell lines. (−)-Epigallocatechin gallate (EGCg) induces apoptosis effectively, while the induction is very weak by (+)-catechin, which lacks a gallolyl group, suggesting a structure-function relationship in apoptosis-inducing activity. To highlight the importance of phenolic hydroxyl groups in association with a catechin skeleton, we examined several polyphenolic compounds derived from tea catechins for apoptosis-inducing activity in human histiolytic lymphoma U937 cells.

U937 cells were obtained from the Health Service Research Resources Bank, Osaka, Japan, and cultured in 10% fetal bovine serum in RPMI 1640 medium (Ikawa Glass Co., Ltd., Chiba, Japan) with 50 μM penicillin, 50 μg/ml streptomycin, 2.5 μg/ml amphotericin B, and 50 μg/ml gentamycin at 37°C under 5% CO2. A caspase inhibitor Z-Asp-CH2-DCB was obtained from the Peptide Institute, Inc., Osaka, Japan. Hoechst 33342 (bischizimide H 33342 Fluorochrome) was obtained from Calbiochem-Novabiochem. Co., CA, USA. SYBR Green was obtained from Molecular Probes, Inc., OR, USA. (−)-Catechin and EGCg were obtained from Funakoshi Co., Ltd., Tokyo, Japan. Theaflavin and theaflavin digallate were prepared as described previously. Preparations of theasinensin D and OH-5 were prepared elsewhere.

U937 cells were incubated in a culture medium in the absence or presence of test compounds. For DNA fragmentation analysis, 5 × 10⁴ cells were pelleted by centrifugation and DNA was isolated from the cell pellets as described by Sellins and Cohen. DNA was electrophoresed in 2% agarose gels, stained with SYBR Green, and imaged and calculated using a FluorImager (Molecular Dynamics Japan, Inc., Tokyo, Japan) (Fig. 2 and Table 1). In order to confirm the apoptosis-associated DNA fragmentation, cells were incubated in the presence of test compounds with a caspase inhibitor, Z-Asp-CH2-DCB (200 μM), at 37°C for 16 h, and DNA fragmentation was examined (Fig. 2 and Table 1).

Here, we confirmed the previously reported apoptosis-inducing activity of EGCg and theaflavins by using U937 cells as detected by DNA ladder formation, which was completely inhibited in the presence of the caspase inhibitor (Fig. 2 and Table 1). Apoptotic bodies were also observed (data not shown). Similarly, theasinensin D and OH-5 were found to induce apoptosis in these cells. (+)-Catechin did not show this activity at least up to 400 μM. Analysis by a fluoroimaging analyzer indicated that the degree of DNA fragmentation by theasinensin D was the highest of the compounds tested, representing about 2.5-fold of that with EGCg (Table 1). Comparison with marker DNAs of known sizes (Takara Shuzo Co., Ltd., Shiga, Japan) indicated that the smallest band observed for theasinensin D (Fig. 2) was of about the 180-nucleotide size.

Induction of apoptosis in U937 cells was also confirmed by observation of chromatin condensation, one of the characteristic features of apoptosis. Cells incubated in the presence or absence of test compounds at 37°C for 16 h were stained with Hoechst 33342 and chromatin condensation was examined under fluorescence

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microscopy by excitation at 365 nm. Percentages of cells with chromatin condensation in total cells were calculated from three randomly selected fields of view. The results showed that the percentages (means ±SD) of the number of apoptotic cells with chromatin condensation were 3.8±3.6, 32.3±4.1, 2.7±0.5, 3.1±0.9, and 3.6±1.6%, in the presence of EGCg, theasinensin D, OH-5, theaflavin, and theaflavin digallate, each at a concentration of 100 μM, respectively, and again that theasinensin D was the most potent apoptosis inducer among these compounds. The percentage of apoptotic cells were 57.2, 25.9, and 3.8% in the presence of EGCg at 400, 200, and 100 μM, respectively, and 32.3, 6.1, and 0.8% with theasinensin D at 100, 50, and 25 μM, respectively, showing the concentration-dependence of the activity.

These data indicate that the three-dimensional position of phenolic hydroxyl groups is a very important fac-

![Structures of Catechin-related Compounds](image_url)

**Fig. 1.** Structures of Catechin-related Compounds.

**Table 1.** DNA Fragmentation Induced by Polyphenols Derived from Tea Catechins

<table>
<thead>
<tr>
<th>Z-Asp-CH₂-DCB</th>
<th>Control</th>
<th>Ts D</th>
<th>OH-5</th>
<th>TD₂</th>
<th>Tf</th>
<th>EGCg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative degradation (%)</td>
<td>8.8</td>
<td>0.0</td>
<td>100.0</td>
<td>0.0</td>
<td>37.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Degrees of DNA degradation based on the data given in Fig. 2 were measured by a fluorimaging analyzer. The value for Ts D in the absence of caspase inhibitor Z-Asp-CH₂-DCB is taken as 100%. For abbreviations see Fig. 2.
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References


