In the course of screening for 17α-hydroxylase/C_{17,20}-lyase inhibitors from food ingredients, the methanol soluble fraction of green tea and black tea, which were expected to be rich in catechin and theaflavin content, showed potent inhibitory activity. \((-\beta\)-Epigallocatechin gallate and theaflavin 3-0-gallate with a pirogallol moiety significantly inhibited C_{17,20}-lyase activity on IC{subscript}50 values of 24.5 μM and 11.5 μM respectively. They had potent cytotoxicity against human prostate cancer LNCaP cells (IC{subscript}50 = 28.1 μM and 37.4 μM).

Key words: 17α-hydroxylase/C_{17,20}-lyase (CYP17); \((-\beta\)-epigallocatechin gallate; theaflavin 3-0-gallate; prostate cancer

Prostate cancer is the most frequently diagnosed malignancy in American males and is increasing gradually in Japanese males due to the western food style. The growth of prostate cancer is dependent on male sex hormones such as testosterone and 5α-dihydrotestosterone (DHT). Treatment is primarily aimed at blocking the synthesis and action of androgens, and 17α-hydroxylase/C_{17,20}-lyase (CYP17, EC1.14.99.9/EC4.1.2.30) is a crucial enzyme in the biosynthesis of androgens. It is the microsomal cytochrome P450-dependent monooxygenase and catalyzes both 17α-hydroxylation (17α-hydroxylation) and cleavage of the C17,20-side chain (C_{17,20}-lyase) during the conversion of 21-carbon steroids pregnenolone (human) and progesterone (rat) to the 19-carbon androgens dehydroepiandrosterone and androstenedione respectively (Fig. 1). Androgen deprivation can be achieved surgically or through the use of LH-RH (luteinizing hormone-releasing hormone) analogs in combination with anti-androgen. But the former treatment can be psychologically unacceptable, and the latter can have side effects. Furthermore, these treatment options eliminate only testosterone production from the testes, and not that produced by the adrenal route. Since the human CYP17 of both testicular tissue that can synthesize about 90% of androgen and adrenal tissue that can synthesize about 10% of androgen are the same gene product, a selective inhibitor of CYP17 has become a promising therapeutic target eliminating production of androgens from both the testes and adrenal cortices (the concept of total androgen block). Although many kinds of synthetic inhibitors of CYP17 have been reported, there is no report of an inhibitor derived from natural products, especially a food ingredient. Hence we screened a new CYP17 inhibitor from the methanol extract of various natural sources, including food ingredients, and found that the green tea and black tea extracts had potent CYP17 inhibitory activity. We identified the active components in green tea as catechins and those in black tea as theaflavins. The effect of green tea and black tea against prostate cancer might depend on inhibition of CYP17 and/or a combination of CYP17 inhibition and 5α-reductase inhibition. In this report, we offer a possible explanation as to why green tea and black tea are effective against prostate cancer.

The various green and black teas were extracted with methanol and evaporated. The dried sample was weighed and dissolved in methanol at 10 mg/ml. Inhibitory screening of CYP17 was performed according to Hartmann et al., with some modifications. Briefly, the assay was performed in 250 μl of assay buffer (50 mM sodium phosphate buffer (pH 7.4), 1 mM MgCl₂, 0.1 mM EDTA, and 0.1 mM dithiothreitol) containing 25 μg protein of rat testes microsomal fraction, 0.5 mM NADPH, and 25 μM substrate (progesterone, the 17α-hydroxylase reaction) or (17α-hydroxyprogesterone, the C_{17,20}-lyase reaction) in the presence or absence of the sample (in 5 μl of MeOH). After incubation for 2 h at 37°C, the reaction was terminated by adding 50 μl of 1 M HCl, followed by 1 ml of ethyl acetate. Extraction of
steroids was accomplished by vortex for 1 min and sonication for 1 min. The micro tube was then centrifuged for 5 min at 6,000 rpm. The organic phase of 900 μl was removed and evaporated. The steroids were dissolved in 100 μl of MeOH, and the aliquot, 20 μl, was subjected to HPLC. HPLC analysis of the reaction products, 17α-hydroxyprogesterone and androstenedione, was done using an Inertsil ODS column (4.6 mm × 150 mm) (GL Science, Tokyo) with methanol/water (75:25) at flow rate of 1 ml/min. UV absorbance was monitored at 240 nm. The inhibitory activity of a sample against CYP17 was examined in the corresponding peak area by HPLC. Inhibition (%) of the 17α-hydroxylase reaction was calculated by the area of 17α-hydroxyprogesterone using progesterone as a substrate (Fig. 1). Values stated are the averages of two independent experiments. Inhibition percentages at 200 μg/ml of several kinds of commercial teas such as green tea, black tea, Jasmine tea, and Pu-erh tea were 85 to 95% against both 17α-hydroxylase and C17,20-lyase. An antifungal clinical drug, ketoconazole, inhibited the latter reaction a little more strongly (IC50 = 35.0 μM, C17,20-lyase) than the former reaction (IC50 = 85.0 μM, 17α-hydroxylase). Thus the specific inhibitor of C17,20-lyase can be recognized by this assay with two kinds of substrate properly. This is valuable for evaluating a specific inhibitor without side effects that has no inhibitory activity on the biosynthesis of glucocorticoids (Fig. 1.).

MeOH extracts of green tea and black tea were analyzed by the PDA (MD-910)-HPLC system (Jasco, Tokyo, Japan), and the HPLC pattern showed the typical
Various catechins were dissolved in MeOH and subjected to the CYP17 reaction. The reaction was performed as described in the text using 17α-hydroxyprogesterone as a substrate.

Table 1. Inhibition Activities of Catechins and Related Compounds on CYP17 (C17,20-lyase)

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Catechin ((+)-C)</td>
<td>&gt;200</td>
</tr>
<tr>
<td>(-)-Epicatechin ((-)-EC)</td>
<td>&gt;200</td>
</tr>
<tr>
<td>(-)-Epigallocatechin ((-)-EGC)</td>
<td>58.0</td>
</tr>
<tr>
<td>(-)-Epicatechingallate ((-)-EGCg)</td>
<td>64.0</td>
</tr>
<tr>
<td>(-)-Epigallocatechingallate ((-)-EGCg)</td>
<td>24.5</td>
</tr>
<tr>
<td>Caffeine</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Gallic acid monohydrate</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>35.0</td>
</tr>
</tbody>
</table>

Inhibition of CYP17 by Green Tea Catechins and Black Tea Theaflavins

Various catechins and theaflavins (Nagara Science, Gifu, Japan) often found in green tea and black tea, and examined the inhibitory activity only on C17,20-lyase of CYP17 using 17α-hydroxyprogesterone as a substrate (Fig. 1), because all extracts of an examined tea inhibited the former reaction (17α-hydroxylase) and the latter reaction (C17,20-lyase) at the same extent. (-)-EGCg, (−)-EGC, and (−)-ECg inhibited CYP17 (C17,20-lyase) in a dose-dependent manner, and IC50 values were 24.5, 58.0, and 64.0 μM respectively (Table 1). Theaflavins had more potent inhibitory activity against CYP17 (C17,20-lyase) than catechins. An inhibition potency was theaflavin 3'-O-gallate (IC50 = 11.5 μM) = theaflavin 3'-O-gallate (IC50 = 12.5 μM) > theaflavin 3,3'-di-O-gallate (IC50 = 15.0 μM) > theaflavin (IC50 = 25.0 μM) (Table 2). Therefore, at least a pyrogallol moiety in catechin and theaflavin appeared to be essential to the potent inhibitory activity of CYP17.

Compared to the activity of ketoconazole, (−)-EGCg and theaflavins showed the almost same activity it did, even though ketoconazole inhibited human CYP17 specifically (Tables 1, 2). Although both reaction products (17α-hydroxyprogesterone and androstenedione) can progress to testosterone and so on due to the microsomal crude enzyme, the inhibition mechanisms of (−)-EGCg and theaflavin 3-O-gallate were non-competitive and mixed inhibition against CYP17 (C17,20-lyase) with a substrate (17α-hydroxyprogesterone) in the Lineweaver-Burk plots. Other functional compounds in tea, (−)-C, (−)-EC, and caffeine, showed no inhibitory activity on CYP17 even at 200 μM. Only gallic acid monohydrate and pyrogallol, which are important moieties in the catechin and theaflavin structures for the inhibition activity, likewise showed no inhibitory activity on CYP17 even at 200 μM (Table 1).

Human prostate carcinoma LNCaP (ATCC CRL-1740, 1 x 10^5 cells/ml) cells and human chronic myelogenous leukemia K562 (ATCC CCL-243, 5 x 10^4 cells/ml) were treated with compounds at various concentrations at 37°C under a humidified 5% CO2 atmosphere for 4 d in RPMI 1640 medium supplemented with 10% fetal bovine serum, 50 units/ml of penicillin, and 50 μg/ml of streptomycin (Gibco, Invitrogen, Carlsbad, CA), and cytotoxicity was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide) assay (Chemicon International, Temecula, CA). Percentages of viable cells were calculated as a ratio of the A570 values of treated and control cells (treated with 0.05% MeOH). Values stated are the averages of two independent experiments. (−)-EGCg and theaflavin 3-O-gallate showed cytotoxicity against LNCaP (IC50 = 28.1 μM and 37.4 μM) and K562 (IC50 = 22.2 μM and 11.8 μM) cells. The cytotoxicity of (−)-EGCg and theaflavin 3-O-gallate was the almost same as that of a CYP17 inhibitory activity.

Epidemiological studies suggest that the consumption of green tea may help prevent various cancers, including prostate cancer in humans. Recently it was reported that oral infusion of a polyphenolic fraction isolated from green tea inhibits the growth and progression of prostate cancer in the transgenic adenocarcinoma of the mouse prostate. And 600 mg administration of green tea catechins (a main component is (−)-EGCg, 51.88%) per d is safe and very effective in treating premalignant lesions before prostate cancer development in humans. Black tea also inhibits prostate cancer tumorigenesis, metastasis, and final tumor weight in association with a reduced serum level of DHT. Tea polyphenols and theaflavins are bioavailable as conjugated and free forms in the human prostate, where they might be active in the prevention of prostate cancer after consumption. These inhibition mechanisms of prostate cancer due to catechins and theaflavins are mainly explained through the inhibitory activity of 5α-reductase (Fig. 1), but this is not yet completely certain. In our experiments, (−)-EGCg and theaflavin 3-O-gallate had potent inhibitory activity on CYP17 (C17,20-lyase), almost the same as that of ketoconazole in vitro. (−)-EGCg also had aromatase inhibitory activity, and a 5% dose of green tea extract catechins (polyphenone-60) given to male rats for 2–8 weeks decreased the weights of the testis and prostate gland. This effect might be due not only to the...
inhibitory activity of catechins on aromatase and/or 5α-reductase, but also to the inhibitory activity of catechins on CYP17, because potent synthetic inhibitors of CYP17 also decrease their weights.19) The prevention of echins on CYP17, because potent synthetic inhibitors of reductase, but also to the inhibitory activity of catechins and theaflavins on aromatase and/or 5α-reductase isozymes by tea epicatechin-3′-gallate and penta-O-galloyl-β-D-glucose inhibit rat liver microsomal 5α-reductase activity and the expression of androgen receptor in LNCaP prostate cancer cells. Carcinogenesis, 25, 1109–1118 (2004).

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References