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Inhibition of green tea catechins against the growth of cancerous human colon and hepatic epithelial cells

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Abstract

The ability of (−)-epicatechin (EC), (−)-epigallocatechin (EGC) and (−)-epigallocatechin gallate (EGCG) to inhibit the growth of HCT 116 colorectal and Hep G2 hepatocellular carcinoma cells was examined by MTT and clonogenic assays (CA). The respective catechins inhibited the growth of HCT 116 more strongly than Hep G2. In MTT assay, IC_{50} values of EGC and EGCG against HCT 116 grew smaller on prolongation of the exposure times of the cells to the catechins. In CA, however, these two catechins had IC_{50} values ranging between 7.6 ± 0.4 and 11.2 ± 0.5 μM against the same cells regardless of the exposure times. EC showed much weaker growth inhibitions relative to the two aforementioned catechins.

Keywords: Green tea catechin; (−)-Epigallocatechin; (−)-Epigallocatechin gallate; Antitumor effect; Human cancer cell line; HCT 116; Hep G2; MTT assay; Clonogenic assay

1. Introduction

It has widely been accepted that drinking green tea is associated with a low incidence of human cancer [1]. One of the catechins from green tea, (−)-epigallocatechin gallate (EGCG), has recently been postulated to prevent human cancers by methods involving inhibition of urokinase [2] and telomerase [3] activities, and angiogenesis [4]. Although studies have been reported on the growth inhibitory effect of tea against human cancer cell lines, little has been reported on its inhibitory effect against the HCT 116 colorectal and Hep G2 hepatocellular human carcinoma cells.

In the present study, we have examined the growth inhibitory activities of (−)-epicatechin (EC), (−)-epigallocatechin (EGC) and EGCG (Fig. 1) against these two human cell lines by using MTT assay (MA) and clonogenic assay (CA).

2. Materials and methods

2.1. Chemicals

EC, EGC and EGCG kindly provided by Dr Y. Hara (Mitsui Norin Co., Ltd.) were dissolved in dimethyl sulfoxide (DMSO) and diluted with culture medium to the desired concentrations. MTT (3-(4,

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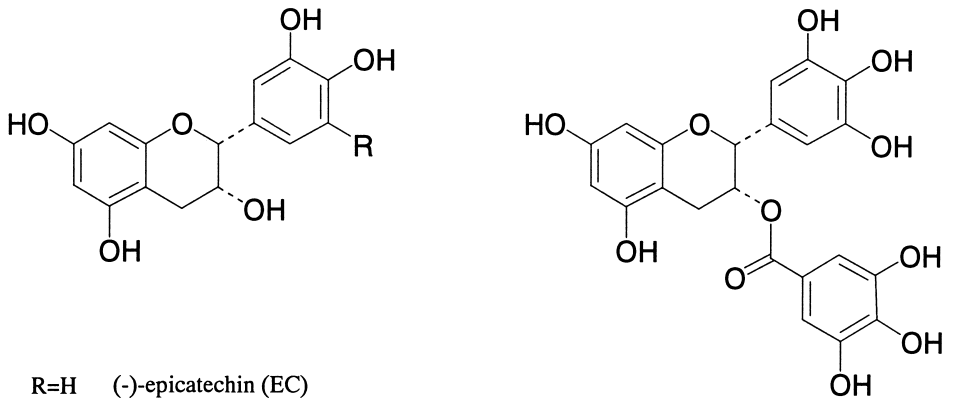

Fig. 1. Strucures of green tea catechins.

5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) was obtained from ACROS ORGANICS (USA). Fetal bovine serum (FBS) was purchased from JRH Biosciences (USA). Giemsa staining solution in glycerol/methanol (50:50) was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan) and diluted 20-fold with phosphate-buffered saline not containing EDTA (PBS(−)).

2.2. Cells

Human colorectal (HCT 116) and human hepatoma (Hep G2) carcinoma cell lines were purchased from ATCC (No. CCL-247 FL) and RIKEN Cell Bank (No. RCB0459), respectively. The former cells were maintained in McCoy's 5A medium (12.0 g/H_2O (1000 ml) and $NaHCO_3$ (2.2 g)) (Nissui, Tokyo, Japan), whereas the latter cells were maintained in Dulbecco's Modified Eagle's medium (10.0 g/H_2O (1000 ml) and kanamycin sulfate (30 mg)) (Nissui, Tokyo, Japan), each medium being supplemented with 10% FBS at 37°C in a humidified atmosphere containing 5% CO_2.

2.3. Cell growth inhibition assays

2.3.1. MA

Cells undergoing exponential growth were suspended in fresh medium at a concentration of 2.5×10^4 cells/ml and inoculated in a 96-well flat-bottomed plate in a volume of 100 μl (2.5×10^3 cells)/well and stabilized by incubation for 1 day at 37°C. Then, 1 μl aliquots of each catechin were added to the wells. After incubation at 37°C for 1 or 3 days, the medium was removed from the wells and the fresh medium was added. The cell survival was determined by the method reported by Mosmann [5]. A solution of MTT, 10 μl (5 mg/ml PBS(−)), was added to each well, and incubation was carried out for 4 h at 37°C. The formazan crystals formed were dissolved by adding 100 μl/well of a solution of 0.04 N HCl and i-PrOH (1:9), and its optical density was measured at 570 nm on a microplate reader (Sjeia AUTO READER III, Sanko Junyaku, Co. Ltd., Japan). Each experiment was performed in triplicate. IC_{50} values were calculated by analysis of the percentage inhibition of each catechin at six concentrations.

2.3.2. CA [6]

Aliquots (100 μl) of 1×10^3 cells/ml were inoculated in a six-well flat-bottomed plate filled with 2 ml of the medium and stabilized by incubation from 1 day at 37°C. Then, 10 μl portions of each catechin solution were added to the wells and incubation was performed at 37°C for 1, 3 or 7 days. After removal of the medium containing the catechin from the wells, the fresh medium was added, and incubation was continued for a total incubation period of 7 days. The medium was removed from the cells, which were successively washed with PBS(−) and immobilized with MeOH. After removing MeOH, the cells were stained with Giemsa staining solution for 30

Table 1
Inhibitory effect of tea catechins against growth of HCT 116 and Hep G2 cancer cells[a]

Cells	Exposure times of cells to catechins (days)	$IC_{50} \pm SD$ (μM)					
		EGCG		EGC		EC	
		MA	CA	MA	CA	MA	CA
HCT 116	1	71.3 ± 9.7	7.7 ± 1.3	64.2 ± 1.0	8.3 ± 1.8	563.7 ± 30.5	44.9 ± 2.9
	3	46.7 ± 5.3	7.6 ± 0.4	46.0 ± 3.1	11.2 ± 0.5	315.2 ± 56.4	35.5 ± 1.5
	7	–	10.0 ± 1.3	–	10.0 ± 1.6	–	22.7 ± 1.1
Hep G2	1	196.4 ± 16.5	56.5 ± 9.1	201.4 ± 6.3	43.6 ± 5.7	>2000	375.8 ± 22.5
	3	141.6 ± 12.3	67.5 ± 3.4	120.6 ± 22.3	52.3 ± 2.9	1006 ± 51.5	295.7 ± 36.2
	7	–	75.3 ± 8.6	–	81.1 ± 6.3	–	225.6 ± 6.3

[a] Assays were performed in triplicate. IC_{50} values are the concentrations at which 50% of the cells are inhibited from growing. In CA, the colony population of the survival cells was counted after a 7 day total period of exposure and subsequent catechin-free incubation. SD, standard deviation.

min, and the stained colony number was counted. Each experiment was performed in triplicate. IC_{50} values were calculated by analysis of the percentage inhibition of each catechin at six concentrations.

3. Results and discussion

The tea catechins were tested for HCT 116 and Hep G2 cell growth inhibition with MA varying the exposure times of cells to each catechin. Their mean IC_{50} values against the cells are listed in Table 1. Furthermore, the values of EGCG and EGC are represented in a bar graph (Fig. 2) with IC_{50} on the vertical axis, a type of catechin on the *x*-axis and the exposure time on the *y*-axis. The IC_{50} values of EGCG and EGC against HCT 116 cells were shifted from 71.3 ± 9.7 and 64.2 ± 1.0 μM to 46.7 ± 5.3 and 46.0 ± 3.1 μM, respectively, on going from 1 day to 3 days of exposure time. The remaining catechin, EC, also showed the same tendency in IC_{50} values as the other two catechins though its values were roughly one-figure larger than those mentioned above. Thus, we tried to estimate IC_{50} values by another method, namely CA. The IC_{50} values of the EGCG and EGC were measured by counting the colony number formed after 7 days of incubation according to the procedure of Puck and Markus [6]. The values of the EGCG and EGC against HCT 116 cells were 10.0 ± 1.3 and 10.0 ± 1.6 μM, respectively, when the cells were exposed to each catechin over a 7 day incubation period. Thus, in order to examine the influence of

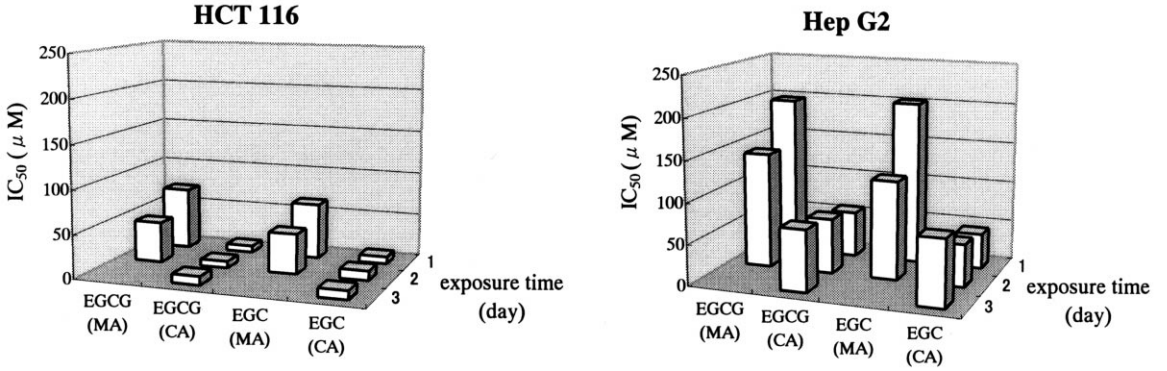

Fig. 2. Graphical representation of inhibitory effect of EGCG and EGC against HCT 116 and Hep G2 cell growth.

the exposure time on IC_{50} values in CA, the HCT 116 cells were incubated first with each catechin for 1 or 3 days and then without the catechin for a further 6 or 4 days, respectively. Unlike in MA, the IC_{50} values of EGCG and EGC were practically unaffected by the exposure times as shown in Fig. 2, i.e. they inhibited the HCT 116 growth at the IC_{50} values ranging between 7.6 ± 0.4 and 11.2 ± 0.5 μM (Table 1). Such a discrepancy in IC_{50} values between these two assays on HCT 116 should be explained by assuming that MA assessed the cell killing due mainly to apoptosis, particularly an early-stage apoptosis, whereas CA measured both apoptosis and cell death occurring several days after exposure to the catechins [7]. Thus, MA eventually underestimated the potency of both EGCG and EGC as a growth inhibitor of HCT 116. We infer that EC might have inhibited the cell growth in a somewhat different mode from that for the other two catechins in CA, considering that the IC_{50} values of EC grew smaller with prolongation of the exposure times.

As compared with the results in HCT 116, none of the catechins markedly inhibited the cell growth of Hep G2 though there was a correlation similar to the case of HCT 116 between the IC_{50} values of EGCG and EGC obtained by the two assays (Fig. 2).

In conclusion, EGCG and EGC, both possessing the pyrogallol structure, are remarkable inhibitors against HCT 116 cell growth and could be potent candidates for colon cancer therapeutic agents if their pharmacokinetical properties including an oral absorbability and a plasma stability are improved by chemical modification, such as prodrug formation.

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