Inhibitory Effect of Cancer Cells Proliferation from Epigallocatechin-3-"O"-gallate

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Abstract
Epigallocatechin-3-"O"-gallate (EGCG), the major polyphenol of green tea and a functional food ingredient/nutraceutical with health-promoting properties. However, its anti-cancer activities on various cancer cells are still little information. Here, we show that anti-cancer activities of the EGCG were evaluated using apoptosis assays analyzed by flow cytometry. The inhibitory activities of proliferation in MPC-11, Caco-2, and MCF-7 cells, values were 66.2, 60.3, and 74.8% at 10 μM, respectively. In addition, in an flow cytometry assay on the MPC-11, Caco-2, and MCF-7 cells, the EGCG showed a cell apoptosis effect on cancer/tumor in vitro model. Our results indicate that EGCG has anti-cancer activities against human lung cancer cells through inducing cell cycle arrest, DNA damage and activating mitochondrial signal pathway. These results indicate that EGCG effectively inhibits in vitro tumor growth by inducing apoptosis of cancer cells.

Keywords: EGCG, cancer/tumor, cell apoptosis, flow cytometer


1. Introduction
Green tea is one of the most widely consumed beverages in the world and its probable health benefits have been the subject of considerable attention [1]. About three billion kilograms of tea is produced and consumed yearly [2]. Epigallocatechin-3-"O"-gallate (EGCG), the major bioactive polyphenolic compound in tea, has been known to possess anticancer, antioxidant, and anti-inflammatory activity [1,3]. Also, EGCG and other tea polyphenols have been shown to prevention/protective effect of various cancer or tumor of the skin, lung, oral cavity, esophagus, stomach, small intestine, colon, bladder, liver, pancreas, prostate and mammary glands [4,5,6]. In addition, EGCG is relatively safe [1,6], and green tea extract containing 60% EGCG has been approved by the US Food and Drug Administration as the first botanical drug [7].

Apoptosis is a naturally occurring process of programmed cell death. In general, drug-induced apoptosis is a major reason for treatment of cancer, and some signal pathways are involved in the process [8]. The intensive efforts and substantial advances that have occurred through focusing on improving treatments, the efficacy of cancer therapies, especially for late-stage disease, remains poor overall, and cancer is still a leading cause of death worldwide [9]. Since the idea of cancer chemoprevention was introduced by Sporn [10] and Wattenberg et al., [11] the hope for overcoming cancer has started to change from one of treatment to one of prevention. The World Health Organization (WHO) indicates that one-third of all cancer deaths are preventable and that diet is closely linked to cancer prevention [12]. In this study, we investigated the effect of green tea polyphenol EGCG on various cancer cell lines.

2. Materials and Methods

2.1. Materials

EGCG, 3-[4,5-dimethy-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay kit were procured from Sigma Aldrich (St. Louis, MO, USA). Culture plates and culture dishes were purchased from Nunc, Inc. (North Aurora Road, IL, USA). Dulbecco’s modified eagle’s medium (DMEM), Roswell Park Memorial Institute (RPMI), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Hyclone (Logan, UT, USA). All other reagents were of the highest grade available commercially.

2.2. Cell Culture

MPC-11, Caco-2, and MCF-7 cell line obtained from American Type Culture Collection (ATCC, Rockville, MD, USA), were cultured in DMEM and RPMI supplemented with penicillin, streptomycin and 10% heat-inactivated FBS in 5% CO₂, 95% air and humidified atmosphere at 37°C.

2.3. MTT Assay

For evaluating the cytotoxicity of EGCG, the cells were harvested using phosphate buffered saline (PBS)
containing 0.15% trypsin and 0.08% EDTA. Cells were incubated in well plates at a density of 5 x 10^5 cells/well. MTT solution was added to each well. Following incubation for 4 h at 37°C in 5% CO₂, the supernatant was removed. The medium was washed by PBS twice, and the formazan crystals produced in viable cells were solubilized in 200 μL DMSO. The absorbance was measured using a microplate reader (Tecan Trading AG, Männedorf, Switzerland) at 550 nm. All experiments were performed with three replicates [13].

2.4. Measurement of Cell Apoptosis

For sub-G1 and cell cycle analysis, B16 cells were suspended in ethanol with 0.5% Tween-20 and left for 24 h at 4°C. The cells were then harvested by centrifugation and resuspended in 1.0 mL of phosphate-buffered saline containing 0.05 mg/mL of PI and 10 μg/mL of RNase A, and incubated at 37°C for 30 min. Analysis of apoptotic cell death was performed by measuring the hypodiploid DNA contents using a flow cytometer (FACS-Caliber; Becton Dickinson; Franklin Lakes, NJ, USA). The cells in the sub-G1 population were considered apoptotic cells, and the percentage of cells in each phase of the cell cycle was determined [14].

2.5. Statistical Analysis

Statistical analyses were performed 3 times for all the experiments. The data are expressed as the mean ± one standard error of mean (SEM). Statistical analyses were assessed by Student’s t-test for paired data. Graph Pad Prism software version 4.00 (Graph Pad Software Inc., San Diego, CA) was used. Significant differences (p < 0.05) between the mean values of the triplicate samples were determined for various assays.

3. Results and Discussion

The effect of EGCG that the viability of three kinds of cancer/tumor cells were examined by MTT assay. MPC-11, Caco-2, and MCF-7 cells were treated with EGCG at various concentrations (0, 0.01, 0.1, 1.0, 5.0, and 10.0 μM, respectively) for 18 h. As shown in Figure 1 (A, B, C), EGCG at up to 0.01 μM showed cytostatic activity against MPC-11, Caco-2, and MCF-7 cells proliferation, and Caco-2 cells inhibitory effect was slightly better than that of MPC-11, and MCF-7. We further investigated the underlying basis of the proliferation-inhibiting effects of EGCG. Cell apoptosis analysis showed the distribution of apoptotic cells throughout the cell cycle, as shown in Figure 2 (A, B, C), where the percentage of apoptotic cells in non-treated cells was 7.49%. The percentages of apoptotic cells in cultures treated with EGCG were 3.23, 4.41, and 4.41%, respectively. Cancers or/and tumors may be reduced by inhibiting cell growth or increasing apoptosis.

The activity of tea and tea polyphenols on the inhibition of skin tumorigenesis has been widely studied. Early reported that the oral administration of green tea polyphenols (GTP) reduced UVB-induced skin tumor incidence, tumor multiplicity and tumor growth in SKH-1 mice [15]. There was also reduced expression of the matrix metalloproteinases (MMP)-2 and MMP-9, CD31, vascular endothelial growth factor (VEGF) and proliferating cell nuclear antigen in the GTP treated group. Additionally, there were more cytotoxic CD8 (+) T cells and greater activation of caspase-3 in the tumors of the orally administered GTP group indicating the apoptotic death of the tumor cells [15]. Recently, EGCG was found to suppress Wnt signaling in invasive breast cancer cells [16]. Green tea or EGCG exhibited chemopreventive action on 7,12-dimethylbenz[a]anthracene-induced mammary carcinogenesis only when given in the postinitiation stage, and the effect was not dose dependent.
In addition, green tea ingestion markedly increased the mean latency of tumors and reduced the tumor burden and the number of invasive tumors in rats with 7,12-dimethylbenz[a]anthracene induced mammary carcinogenesis [17]. In a case-control study conducted in China, drinking green tea was found to decrease the risk of esophageal and gastric cancers [18]. It has been reported that small intestinal tumorigenesis was inhibited in a dose-dependent manner by oral administration of EGCG which was accompanied by increased levels of E-cadherin and decreased levels of nuclear β-catenin, c-myc, phospho-Akt, and phospho-ERK1/2 in small intestinal tumors in Apc (min/+) mice [19,20].

Figure 2. Cell death and cycle of MPC-11 (A), Caco-2 (B), and MCF-7 (C) cells using flow cytometry analysis. Various cells were incubated with 0.01, 0.1, 1, 5, and 10 μM of EGCG for 24 h. The results are shown as percentages of control samples. Data are presented as the mean ± S.E.M. (n = 3) for three independent experiments. Significance was determined by Student’s t-test. # *p < 0.05.

4. Conclusions

The current study demonstrated that green tea polyphenol EGCG has an inductive effect on various cancer cells. In light of the fact that natural products have been used as traditional medicines and now are potential sources of new drugs or nutraceuticals, our study to verify inhibitory effects of cancer cell from EGCG and its less toxicity and better tolerability, further consideration of the therapeutic applications.

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References


