Inhibitory Effect of Epigallocatechin-3-gallate on Growth and Invasion in Human Biliary Tract Carcinoma Cells

Moriatsu Takada, M.D., Ph.D., Yonson Ku, M.D., Ph.D., Kazuto Habara, M.D., Tetsuo Ajiki, M.D., Ph.D., Yasuyuki Suzuki, M.D., Ph.D., Yoshikazu Kuroda, M.D., Ph.D.

First Department of Surgery, Kobe University School of Medicine, 7-5-2, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

Published Online: March 1, 2002

Abstract. Based on recent evidence that tea consumption contributes to a decreased incidence of human carcinomas, a number of investigators have focused on the mechanisms of cancer prevention by tea extracts, especially green tea polyphenols. Epigallocatechin-3-gallate (EGCG) is a representative polyphenol that inhibits the activity of the cyclin-dependent kinases of cdk2 and cdk4. This suggests that EGCG may exert its growth-inhibitory effects through modulation of G₁ regulatory proteins such as cdk2 and cdk4. The human biliary tract carcinoma cells (TGBC-2, SK-ChA-1, and NOZC-1) were treated with different doses of EGCG (0, 25, 50, 100, and 200 μM) for 48 hours in cell medium. Cell proliferation was analyzed by WST-1 colorimetric assay. For the cell-invasion analysis, the cells were incubated with 100 μM of EGCG for 2 hours. The cells were then added into a Matrigel-coated Cell Insert. After incubation at 37°C for 24 hours, the cells visible through the Matrigel were counted under the microscope. All human biliary tract cancer cells studied showed a significant suppression of cell growth by EGCG treatment in a dose-dependent manner (27.2%, 16.0%, and 10.1%, in TGBC-2, SK-ChA-1, and NOZC-1, respectively, at the dose of 200 μM). Epigallocatechin-3-gallate treatment also produced a significant suppression of invasive ability of the carcinoma cells (12.6%, 11.2%, 7.9%, in TGBC-2, SK-ChA-1, and NOZC-1, respectively, at a dose of 100 μM). These data indicated that EGCG might be a potent biological inhibitor of human biliary tract cancers, reducing their proliferative and invasive activities.

Materials and Methods

Epigallocatechin-3-gallate

A purified preparation of EGCG (> 98% pure) that was isolated from green tea using reverse-phase high-performance liquid chromatography (HPLC) was obtained from Food Research Laboratories, Mitsui Norin Co. Ltd. (Shizuoka, Japan). The purified EGCG dissolved in phosphate-buffered saline (PBS; 50 mM, pH 7.4) was used for the treatment of cells.

Human Biliary Tract Cancer Cell Culture

The human biliary tract cancer cell lines (TGBC-2, SK-ChA-1, NOZC-1) were studied. Gallbladder cancer cell line, TGBC-2, and bile duct cancer cell line, SK-ChA-1 were kindly donated by Dr. Takeshi Todoroki (Department of Surgery, Institute of Clinical Medicine, University of Tsukuba, Japan) [9]. Another human gallbladder cancer cell line, NOZC-1, was given by Dr. Seishi Nagamori (Jikei University School of Medicine, Tokyo, Japan) [10]. The cells were maintained with Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum at 37°C in an atmosphere of 5% CO₂ in humidified chambers as described elsewhere [9, 10].

Cell-proliferation Analysis

The cell proliferations were analyzed by WST-1 colorimetric assay. The cells (70% to 80% confluent) were treated with different doses of EGCG (0, 25, 50, 100, and 200 μM) and cultured in a 96-well microplate for 2 days. Then, 10 μl of WST-1 (Takara Biomedicals, Shiga, Japan) has added to the cells and incubated for 2 hours and the absorbances (450/630 nm) were measured.
Fig. 1. Effect of epigallocatechin-3-gallate (EGCG) on cell proliferation of human biliary carcinoma cells. Treatment with EGCG (25 to 200 μM for 48 hours) shows dose-dependent inhibition of cell growth. Asterisks denote the statistical differences compared to no EGCG treatment (p < 0.01).

Fig. 2. Effect of EGCG on cell invasion of human biliary carcinoma cells. Preincubation with EGCG (100 μM for 2 hours) shows inhibition of cell invasion through Matrigel basement membrane (filled bars) compared to no EGCG treatment (open bars). Asterisks denote the statistical differences between the groups (p < 0.01).

using ELISA plate reader. We measured three samples in each data point, and repeated three independent experiments. The data point is expressed as the arithmetic mean ± SEM using the three samples in a representative experiment among the three experiments.

**Cell-invasion Analysis**

Cells were preincubated in 100 μM of EGCG for 2 hours, after which 500 μl/well of serum-free medium was added to the plate, and 200 μl/well of the cell suspension at 5 × 10⁴/ml was added to a Matrigel-coated Cell Insert (8 μm pore size, 25 μg/well coated; Becton Dickinson Labware, Tokyo, Japan). The chambers were incubated at 37°C in 5% CO₂ in air for 12 hours. After incubation, the cells on the upper surface of the filter were completely wiped clean with a cotton swab and monitored visually under the microscope (AX80T, Olympus, Tokyo, Japan). The filters were then fixed in methanol and stained with hematoxylin to identify the cells that penetrated the Matrigel basement membrane matrix. Various areas of the lower surface were scanned (Fujix Photograb-2500 for Macintosh SH-25/M, Tokyo, Japan), and the cells were counted in hexaplicate under software analysis (Mac SCOPE, Mitani Co., Tokyo, Japan).

**Statistical Analysis**

The data are presented as arithmetic means ± standard error of the mean (SEM). Statistical comparisons between groups were performed by Student's t-test. The difference was considered significant at p < 0.01.

**Results**

**Effects of EGCG on Cell Proliferation of Human Biliary Cancer Cells**

As shown in Figure 1, EGCG treatment (25 to 200 μM for 48 hours) resulted in the inhibition of cell growth in a dose-dependent manner. Cell growth of TGBC-2 was significantly inhibited with 50 μM of EGCG. Addition of 100 μM and 200 μM resulted in the same degree of cell inhibition, indicating that the maximal inhibition of cell proliferation by EGCG reached a plateau. Similarly, cell growth of NOZC-1 was inhibited in the same way as TGBC-2. Addition of 50 to 200 μM of EGCG showed almost the same inhibitory effect on these two types of gallbladder cancer cells. By comparison, cell growth of bile duct cancer cells, SK-ChA-1 was linearly decreased in a dose range of 0 to 200 μM. In comparing the three cell lines, addition of 200 μM of EGCG inhibited the cell growth to 27.2% in TGBC-2, 16.0% in SK-ChA-1, and 10.1% in NOZC-1, showing the most prominent effect on NOZC-1. These inhibitory effects on human biliary tract carcinoma cells were confirmed by direct microscopic observations.

**Effects of EGCG on Cell Invasion of the Human Biliary Cancer Cells**

Preincubation for 2 hours at the dose of 100 μM of EGCG showed significant inhibition of cell invasion through Matrigel, irrespective of the cell line determined. The number of invaded TGBC-2 cells was 5.33 ± 1.89 with EGCG, whereas it was 42.17 ± 6.57 without EGCG. This effect was analogously reproduced in the other cell lines analyzed; SK-Ch-A-1 showed 4.50 ± 1.26 with EGCG and 40.33 ± 6.75 without EGCG. The effect of EGCG on cell invasion was the most prominent in NOZC-1; 3.83 ± 1.95 with EGCG and 48.67 ± 9.98 without EGCG. The relative inhibitory ratio was 12.6% in TGBC-2, 11.2% in SK-ChA-1, and 7.9% in NOZC-1 (Fig. 2).

**Discussion**

Biliary tract carcinomas show several disorders in TGF-β inhibitory pathways and TGF-β receptors [5]. Gohongi et al. reported that TGF-β inhibits the proliferation of gallbladder carcinoma cells [11]. Recent studies have demonstrated that expression of p27 and p21 is decreased and that cyclin D1 is overexpressed in