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The effect of quercetin on cell cycle progression and growth of human gastric cancer cells

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Quercetin, a flavonoid, is found in many plants, including edible fruits and vegetables. We examined the effects on cell growth of human malignant cells derived from the gastrointestinal tract and on cell cycle progression. Quercetin markedly inhibited the growth of human gastric cancer cells and the IC_{50} value was 32–55 μM. DNA synthesis was suppressed to 14% of the control level by the treatment with 70 μM quercetin for 2 days. Furthermore, quercetin blocked cell progression from the G_1 to the S phase.

Quercetin; Cell cycle; (Human gastric cancer cell)

1. INTRODUCTION

Quercetin, a flavonoid found in many plants, is widely distributed in edible fruits and vegetables. Humans regularly consume foods containing quercetin not only in its free form, but also in the β-glycoside form, such as rutin and quercitrin. When quercetin was administered orally, it was poorly absorbed from the digestive tract and did not have a great influence on the organs except the gastrointestinal tract. A proportion of the quercetin administered was degraded by the intestinal microflora and the remainder was excreted in feces in an unchanged form [1–2].

Recently quercetin was found to inhibit the cell growth of leukemia cells, Ehrich ascites tumor cells [3], and NK/Ly ascites tumor cells [4]. To clarify the mechanism of anti-tumor effects, we examined its effect on the cell growth of four human gastric cancer cell lines, as well as on the cell cycle.

2. MATERIALS AND METHODS

2.1. *Cell line and chemicals*

Quercetin was purchased from Wako Pure Chemical Industries, Osaka. In this study, four human gastric cancer cell lines, HGC-27 [5], NUGC-2 [6], MKN-7, and MKN-28 [7] were used. Cells were cultured in RPMI 1640 (Nissui) supplemented with 10% fetal calf serum and incubated at 37°C in a humidified atmosphere containing 5% CO_2. Quercetin was dissolved in 0.25% dimethyl sulfoxide (DMSO) and diluted to its final concentration in each culture dish.

2.2. *Measurement of cytotoxicity*

Cells were plated at a density of 4×10^4 cells/2 ml medium in 35 mm diameter dishes. The various concentrations of quercetin were added at the inoculation of cells, or at the 2nd day after the inoculation of cells. Control cells were exposed to DMSO at the same concentration as the other dishes. The culture was continued without medium change. The number of viable cells were measured by the Trypan blue dye exclusion test. The values represent means ± SD. Data were analyzed using a two-tailed Student's t-test.

2.3. *Measurement of DNA synthesis*

DNA synthesis was assayed at 1, 3, 12, 18, 24, 36, and 48 h after treatment with quercetin. HGC-27 cells were incubated for 1 h with 10 μCi of [^{3}H]thymidine. The cells were washed with 2 ml of phosphate-buffered saline, treated with 2 ml of 5% trichloroacetic acid (TCA), and kept overnight or longer in a wet chamber. Cells were washed with 2 ml of 5% TCA and solubilized with 0.8 ml of 1% sodium dodecyl sulphate. The radioactivities of aliquots were counted in a liquid scintillation spectrometer. Data were converted to dpm per μg cellular protein.

Protein concentrations were determined by a modification of the method of Lowry et al. [8]. The results were expressed as percent inhibition, compared with the result of the control experiment in which quercetin was absent.

2.4. *Analysis of cell cycle progression*

Cells were plated at a density of 1×10^5 cells/5 ml medium in 55 mm diameter dishes. Quercetin at a concentration of 70 μM was added after 2 days. In the preliminary data we found that the effect of quercetin on the cell growth diminished with time. Thus, the medium with quercetin was changed every 8 h for 24 h (see fig.3, 1–3). After this incubation, the cells were transferred to the medium without quercetin in some experiments, in order to analyze in which phase quercetin has an effect (see fig.3, 4–9). In order to synchronize cells to the G_1 phase, cells were plated at a density of 1×10^5 cells/55 mm diameter dish and cultured in the above medium containing 10% FCS for 24 h. The cells were then washed once with PBS and incubated in the same medium containing 0.5% FCS for 72 h. After this incubation, more than 90% of the cells were in the G_1 phase. The cells were then transferred to the medium containing 10% FCS to initiate progression of the cell cycle. One group of cells was incubated in the above medium without quercetin (see fig.4, A_1–A_5) and the other group was incubated in the above medium with 70 μM quercetin to

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analyze the effect of quercetin on the cells in the G_1 phase (see fig.4, B_1–B_5). The G_1-enriched cells by serum starvation shifted to the S phase and the G_2/M phase as a synchronized cell population in the medium containing 10% FCS for 19 h. These S- and G_2/M-enriched cells were incubated in the above medium with 30 μM quercetin to analyze the effect of quercetin on the cells in the S phase and the G_2/M phase (see fig.5).

2.5. *Flow cytometric analysis*

Cells were removed from culture plates by brief trypsinization and were spun down at 750 rpm for 5 min. After the cell pellets were washed with 3 ml of ice-cold Tris buffer (1.2% Tris, 0.6% NaCl, pH 7.6), they were suspended in 1 ml of ice-cold 0.1% Triton X-100 in 0.3 M NaCl/0.01 M glycine and 0.2 ml of 1% RNase solution was added. The DNA of these cells was stained with 0.1 ml of ethidium bromide solution (0.2 mg/ml). The DNA content was analyzed by using a flow cytometer FACStar (Becton Dickinson Facs System).

3. RESULTS

3.1. *Effect on progression of the human gastric cancer cells*

Fig.1 shows the growth curve of HGC-27 cells in the presence of various concentrations of quercetin. A dose-dependent inhibition of growth was observed over 10 μM (3.4 μg/ml). At 48 h after the addition of quercetin, the growth of the cells decreased to about 70%, 23%, and 10% of the control level by the treatment with 10, 30, and 70 μM of quercetin, respectively. Cell numbers were gradually reduced to below the level of the inoculum at quercetin concentrations of over 70 μM. IC_{50} values of four human gastric cell lines were 32–55 μM. HGC-27 cells, which we used mainly in this study, and NUGC-2 are poorly differentiated adenocarcinoma cells, and the other two cell lines, MKN-28 and MKN-7 cells, are well-differentiated adenocarcinoma cells. There was no difference in the sensitivity to quercetin among these types of cells (table 1).

3.2. *Effect on DNA synthesis*

Quercetin suppressed DNA synthesis in a dose-dependent manner. DNA synthesis was suppressed to about 64%, 48%, and 14% of the control level with 10, 30, and 70 μM quercetin, respectively, at 12–18 h after the addition of quercetin. Suppression of DNA synthesis was observed as early as 1 h after addition of quercetin. With 10 μM of quercetin, DNA synthesis began to increase after 12 h and recovered to about 90% of the control level at 48 h. With 30 and 70 μM of quercetin, DNA synthesis began to increase after 18–24 h (fig.2).

Table 1

Effect of quercetin on the proliferation of four gastric cancer cell lines

Cell line	IC_{50} (μM)
HGC-27	52
NUGC-2	32
MKN-28	35
MKN-7	55

Various concentrations of quercetin were added simultaneously at the inoculation of cells. The culture was continued for 4 days. The IC_{50} of each cell line was calculated

3.3. *Effect on cell cycle progression*

As shown in fig.3, during the first 24 h, the cytokinetic alterations induced by quercetin were characterized by marked G_2/M phase evacuation and S phase reduction. After removal of quercetin, cells moved through the S, G_2, and M phase as a synchronized cell population. These results suggest that quercetin arrests these cells at the G_1 or early S phase. As shown in fig.4, serum starvation resulted in enrichment of HGC-27 cells in the G_1 phase of the cell cycle. When these cells were supplemented with 10% FCS, progression into the cell cycle was initiated. Cells in the G_1 phase decreased and cells in the S phase gradually increased during 16–20 h. At 20 h, the proportion of cells in the S phase markedly increased. Many cells shifted to the G_2/M phase during 20–24 h and accumulated again in the G_1 phase at 36 h (fig.4, A_1–A_5).

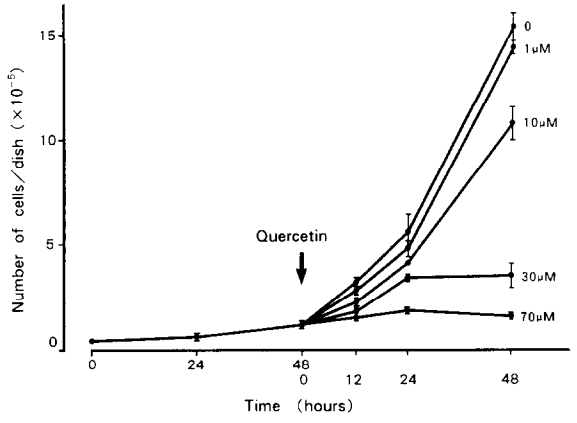

Fig.1. Effect of quercetin on the growth of HGC-27 cells. Various concentrations of quercetin were added on the 2nd day after inoculation of cells. The values represent means ± SD. The arrow indicates when quercetin was added.

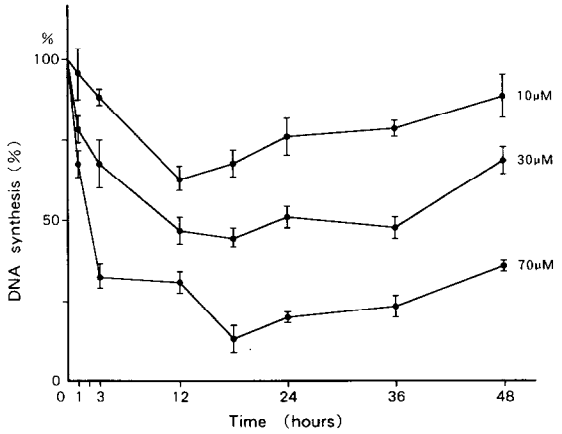

Fig.2. Effect of quercetin on DNA synthesis in HGC-27 cells. Various concentrations of quercetin were added on the 2nd day after the inoculation of cells. After 1, 3, 12, 18, 24, 36, 48 h, DNA synthesis was assayed as described in section 2. The values represent means ± SD.

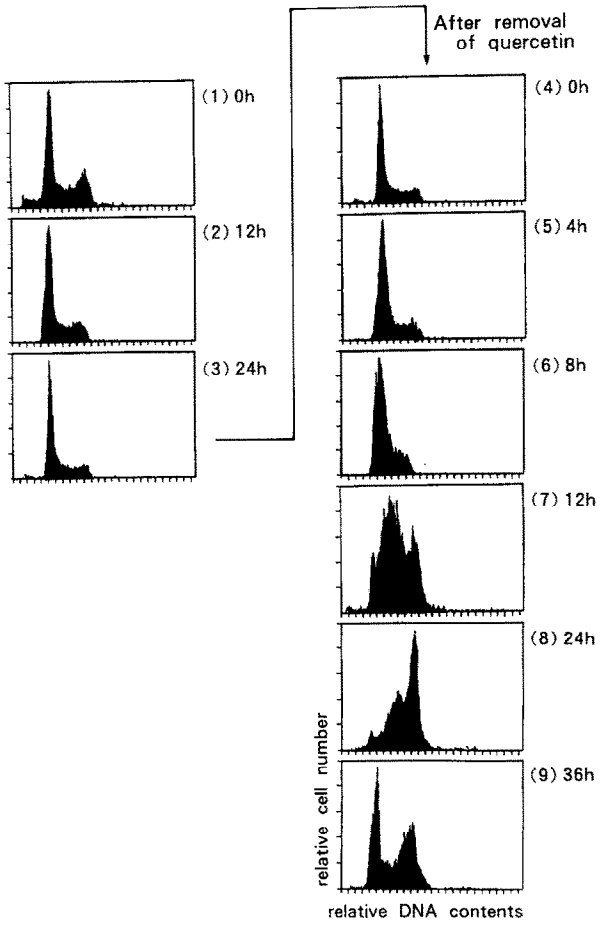

Fig.3. DNA histograms of HGC-27 cells. Cells were plated at a density of 1×10^5 cells/dish and cultured in media containing 70 μM of quercetin. The medium with 70 μM of quercetin was changed every 8 h (1–3). After this incubation, cells were transferred to the medium without quercetin (4–8).

When 70 μM of quercetin was added to the G_1-enriched cells, it inhibited progression of the cells into the S phase for 24 h (fig.4, B_1–B_4). After 36–48 h, the cells began to progress into the S phase (fig.4, B_5). This progression was delayed for 20 h compared to the control cases.

When 30 μM of quercetin was added to the S- and G_2/M-enriched cells, it did not inhibit progression of the cells into the G_2/M phase and the G_1 phase (fig.5). Cells in the S phase and the G_2/M phase decreased and the proportion of cells in the G_1 phase gradually increased. However, cells in the G_1 phase were not found to shift to the S phase.

4. DISCUSSION

Various biochemical effects of quercetin have been shown in numerous studies. Regarding its mutagenicity, quercetin was shown to exhibit mutagenic activity in both bacterial [9–11] and mammalian cell systems [12–14]. Although some mutagens show carcinogenic activity and their effects overlap with some carcinogens, mutagenicity has been proven to not necessarily be related to carcinogenicity. In fact, the carcinogenicity of quercetin has been extensively studied, but could not be confirmed [15–20], with one exception [21]. Nishino et al. [22] and Kato et al. [23] showed that quercetin inhibited the cell growth-promoting effect of teleocidin or 12-*O*-tetradecanoylphorbol-13-acetate on skin papilloma formation in mice. These results indicate that quercetin is not carcinogenic despite its mutagenicity.

Quercetin was shown to inhibit the synthesis of DNA, RNA, and protein in Ehrich ascites tumor cells [24]. This is in agreement with our results which showed that quercetin markedly decreases the synthesis of DNA. The time course of change in DNA synthesis in gastric cancer cells treated with quercetin was found to correlate well with that of the decrease of the S phase population by flow cytometric analysis (fig.3).

It is thought that in the cell cycle phases, the G_1 phase is the major site for the control of animal cell proliferation and the major difference between normal cells and malignant tumor cells depends on the G_1 phase. We demonstrated that quercetin induced a specific G_1 arrest in human gastric cancer cells.

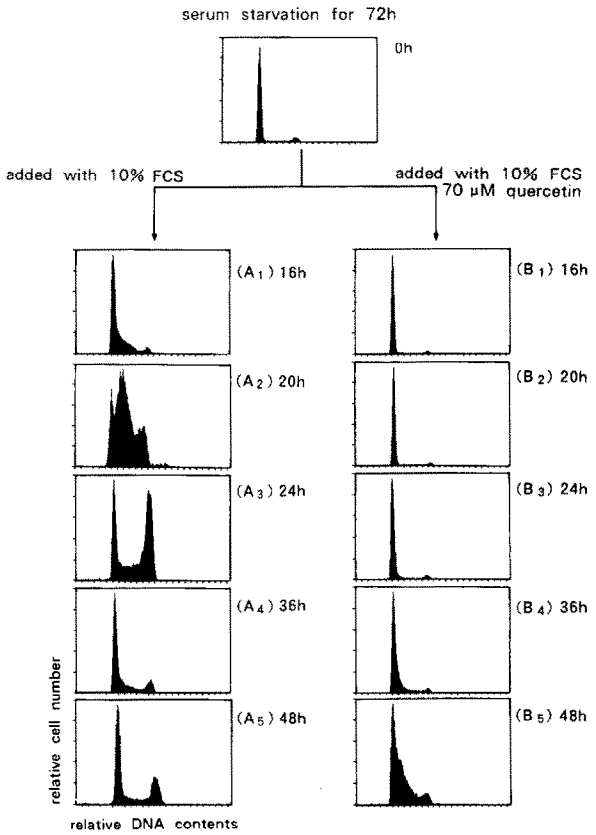

Fig.4. DNA histograms of HGC-27 cells after serum starvation. To synchronize cells to the G_1 phase, cells were incubated in the same medium containing 0.5% FCS for 72 h (0 h). The control group of cells was transferred to the medium containing 10% FCS to initiate progression (A_1–A_5). The other group of cells was transferred to the medium containing 10% FCS and 70 μM quercetin (B_1–B_5).

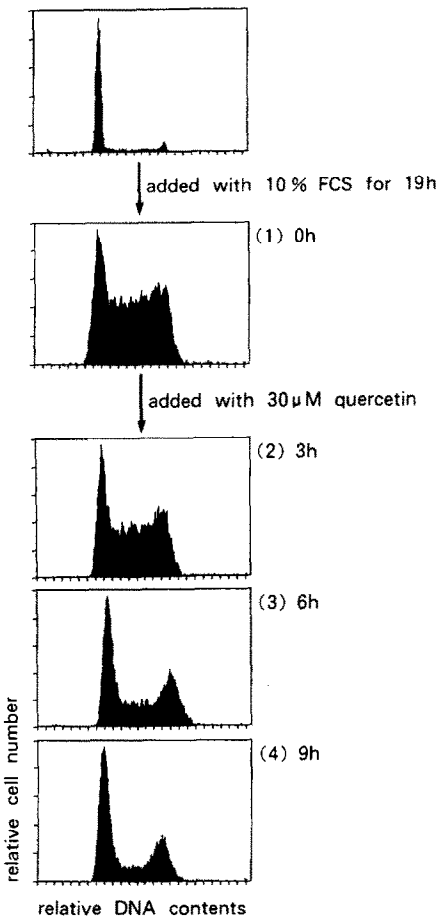

Fig.5. DNA histograms of HGC-27 cells after serum starvation. To synchronize cells in the S phase and the G_2/M phase, cells were incubated in the same medium containing 10% FCS for 19 h after serum starvation (1). These cells were transferred to the medium with 30 μM quercetin added (2–4).

Most cells were apparently arrested at the end of the G_1 phase by the treatment with quercetin, and after removal of quercetin, moved through the subsequent S, G_2, and M phases as a synchronized cell population. The results of our experiments on short time exposure to quercetin demonstrated that quercetin-induced cell cycle arrest was reversed by the cell wash. Since, in these studies, however, exponentially growing cells were used for the analysis, the precise properties of quercetin-induced cell arrest have not been characterized. We therefore synchronized the growth of HGC-27 cells by serum starvation, and analyzed the effects of quercetin on these cells (figs 4,5). The results indicate that quercetin had no effect on the cell cycle progression in the S phase and the G_2/M phase, and the growth inhibitory effect of quercetin was the result of the arrest in the G_1 phase of the cell cycle.

Human beings are exposed to quercetin continuously and it has been estimated that the average person is exposed to about 50 mg 'quercetin equivalents' per day [25]. This suggests that it is a potent anticancer agent in man. In addition, the frequent intake of fruits and vegetables which contain quercetin may be a successful cancer preventive measure. Quercetin may be important from the point of view of the epidemiology concerning malignant tumors of the gastrointestinal tract.

Although we found that quercetin induces a specific G_1 arrest in the human gastric cancer cell, the biochemical mechanism of this G_1 block is not yet known. Further investigation to clarify the mechanism of the cytotoxic action of quercetin and the regulating mechanism of the cell cycle progression by using quercetin, is now in progress.

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