Original article

In-vitro effect of a combination of 5-fluorouracil (5-FU) and cisplatin (CDDP) on human gastric cancer cell lines: timing of cisplatin treatment

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Abstract

Background. To date, we have few effective chemotherapeutic agents against advanced and recurrent gastric cancer. 5-Fluorouracil (5-FU) and cisplatin (CDDP) are the most widely used drugs, and their combination has demonstrated favorable outcomes. However, the method (especially the timing) of CDDP administration is not well established.

Methods. We examined the in-vitro effect of a combination of 5-FU and CDDP on four human gastric cancer cell lines: MKN-1, MKN-28, MKN-45, and MKN-74. The cell lines were exposed to 50% of the inhibitory concentrations of 5-FU and CDDP for 72 h and 8 h, respectively. CDDP was applied before, simultaneously with, and after the start of treatment with 5-FU.

Results. When CDDP was applied after 5-FU, the cytotoxic activity against MKN-28, MKN-45, and MKN-74 was significantly potentiated. Against MKN-1, the earlier the initiation of CDDP treatment, the stronger was the cytotoxic effect.

Conclusion. Our results suggest that the cytotoxicity of a combination of 5-FU and CDDP against human gastric cancer cells is both cell-line- and schedule-dependent and is especially affected by the timing of the CDDP treatment.

Key words 5-Fluorouracil · Cisplatin · Gastric cancer

Introduction

5-Fluorouracil (5-FU) and cisplatin (CDDP) are widely used anticancer drugs that act synergistically against human carcinomas, especially those arising in the head and neck [1–3]. However, few chemotherapeutic regimens have been shown to be consistently effective against advanced gastric cancer. Recent clinical trials of

5-FU plus CDDP in patients with gastric cancer demonstrated a favorable outcome, with response rates of 43% to 45% [4–6]. Experimentally, several investigators [7–9] have shown that CDDP enhances the antitumor activity of 5-FU, both in vitro and in vivo. The biochemical mechanisms responsible for this favorable interaction are partly understood. Nevertheless, the method and timing of CDDP treatment remains controversial; a universally accepted schedule for 5-FU and CDDP therapy has not yet been established.

To gain insight into the optimal schedule for treatment with 5-FU and CDDP, we studied the effect of altering the time of CDDP treatment on cells continuously exposed to 5-FU.

Materials and methods

Chemicals

5-FU and CDDP were purchased from Kyowa Hakko (Tokyo, Japan) and Nihon Kayaku (Tokyo, Japan).

Cell culture

Four human gastric cancer cell lines (MKN-1, MKN-28, MKN-45, and MKN-74) were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan). The cells were maintained in RPMI-1640 (Gibco BRL, Gaithersburg, MD, USA) and 0.5% penicillin-streptomycin (Gibco BRL) at 37°C in an atmosphere of 5% CO₂ in a humidified incubator. These culture conditions were used with the following assays.

Determination of drug concentration to inhibit cell proliferation by 50% (IC₅₀)

The cells were cultured for 4 days in the logarithmic growth phase at 37°C in an atmosphere of 5% CO₂ to decide the number of cells to seed. Cells were confluent

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in 4 days when 5000 cells/well for MKN-1, 1000 cells/well for MKN-28, 5000 cells/well for MKN-45, and 4000 cells/well for MKN-74 were added to 96-well microplates (Sumitomo Bakelite, Tokyo, Japan) on day 0. These cells were exposed to various concentrations of 5-FU for 72 h and CDDP for 8 h from day 1. On day 4, a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was performed to evaluate cytotoxicity, as follows [10]. Ten μ l of MTT solution (MTT Kit; Chemicon, Temecula, CA, USA) was added to each well, and the plates were further cultured for 4 h. Then 100 μ l of 0.04 N HCl in isopropanol was added, and the plates were cultured overnight. Thorough mixing was carried out to dissolve the dark blue crystal, and the absorption was measured at a 550-nm wavelength (optical density [OD] 550) with a 630-nm reference wavelength, by a microplate reader NJ-2300; (Nalge Nunc, Tokyo, Japan). The IC 50 of 5-FU (72 h) and CDDP (8 h) for each cell line was determined by plotting the cell proliferation rate (OD 550 at drug application/OD 550 of control) against various drug concentrations.

Drug treatment schedule

The cells were added to 96-well microplates, cultured for 24 h, and exposed to the IC 50 of both 5-FU (72 h) and CDDP (8 h) according to the following schedules (Fig. 1). Schedule A, 5-FU alone (72 h); schedule B, CDDP (8 h) followed by 5-FU (72 h); schedule C, 5-FU and CDDP (8 h) followed by 5-FU (64 h); schedule D, 5-FU (8 h), 5-FU and CDDP (8 h), followed by 5-FU (56 h); and schedule E, 5-FU (16 h), 5-FU and CDDP (8 h), followed by 5-FU (48 h). In schedules D and E, the cells were washed with culture fluid to eliminate CDDP. Then only 5-FU was applied again. Absorption was measured with a microplate reader, by MTT assay, and the cell proliferation rate (OD 550 at drug application/OD 550 of control) was determined. Experiments were performed in triplicate.

Statistics

All data were analyzed by Student's *t*-test. *P* values of less than 0.05 were considered to be statistically significant.

Fig. 1. Schedules of drug treatment. A 5-Fluorouracil (FU) alone (72 h); B cisplatin (CDDP) (8 h) followed by 5-FU (72 h); C 5-FU and CDDP (8 h) followed by 5-FU (64 h); D 5-FU (8 h), 5-FU and CDDP (8 h), followed by 5-FU (56 h); and E 5-FU (16 h), 5-FU and CDDP (8 h), followed by 5-FU (48 h)

Results

Measurement of IC 50 values

The IC 50 values of 5-FU at 72 h and those of CDDP at 8 h were calculated. MKN-28 cells were the most sensitive to 5-FU, and MKN-1 and MKN-45 cells were the most sensitive to CDDP (Table 1).

Cytotoxic effect of 5-FU and CDDP combination

A combination of 5-FU and CDDP was more cytotoxic than 5-FU alone against all four cell lines. The proliferation of MKN-1 cells was most inhibited by schedule B (CDDP [8 h] followed by 5-FU [72 h]); the earlier the time of CDDP treatment, the greater was the cytotoxic effect of 5-FU and CDDP (Fig. 2). In contrast, for the other three cell lines, the later the initiation of CDDP treatment, the greater was the cytotoxic effect of the combination (Figs. 3, 4, 5). Against MKN-45 and MKN-74, in particular, schedule E was much more cytotoxic than schedule D. In MKN-28, schedule E did not significantly inhibit the cell proliferation as compared with schedule D.

Table 1. The drug concentration to inhibit cell proliferation by 50% (IC 50) of each cell line

	IC 50 (μ g/ml)	
	5-Fluorouracil (FU)	Cisplatin (CDDP)
MKN-1	0.63	2.0
MKN-28	0.19	8.0
MKN-45	0.32	2.0
MKN-74	0.34	11.0

Fig. 2. Cytotoxic effect against MKN-1 cells. The cell proliferation rate (%) was lowest for schedule B, followed by C, D, and E. **P* < 0.05

Fig. 3. Cytotoxic effect against MKN-28 cells. The cell proliferation rate (%) was lowest for schedule D, followed by E, C, and B. * $P < 0.05$

Fig. 5. Cytotoxic effect against MKN-74 cells. The order of the cell proliferation rate was $B > C > D > E$. The later the initiation of CDDP treatment, the greater was the combination effect of 5-FU and CDDP. * $P < 0.05$

Fig. 4. Cytotoxic effect against MKN-45 cells. The order of the cell proliferation rate was $B > C > D > E$. The later the initiation of CDDP treatment, the greater was the combination effect of 5-FU and CDDP. * $P < 0.05$

Discussion

Experimentally, two conflicting types of sequence dependence have been demonstrated for the cytotoxic activity of a combination of 5-FU and CDDP. Scanlon et al. [7] found that cytotoxic activity was synergistically enhanced when CDDP was given before 5-FU in A2780 human ovarian cancer cells; the cytotoxicity was 76% for CDDP followed by 5-FU and 59% for the reverse sequence. They proposed that CDDP pretreatment blocked methionine uptake into tumor cells and increased the synthesis of intercellular methionine, thus enhancing the cytotoxicity of 5-FU. This action was also demonstrated in vivo [8], and the sequence of CDDP followed by 5-FU was proposed to be the most effective. However, other groups of investigators [9,11,12] have

reported higher cytotoxic activity when 5-FU was applied before CDDP. Esaki et al. [9] showed that cytotoxicity was augmented when CDDP was given 24 or 48 h after 5-FU treatment in an HST-1 human squamous carcinoma cell line. They suggested that 5-FU inhibited the repair of the DNA interstrand cross-links formed by CDDP and increased the antitumor activity of CDDP. Our previous study [13] of four gastric cancer cell lines also demonstrated no enhancement by the sequence of CDDP followed by 5-FU.

These conflicting results suggest the hypothesis that the cytotoxic effect of a combination of 5-FU and CDDP is not only schedule-dependent but also cell line (perhaps cell type)-dependent. Kano et al. [14] demonstrated the cell line-dependence of paclitaxel and cisplatin, using four different human carcinoma cell lines: nonsmall cell lung cancer, A549; breast cancer, MCF7; ovarian cancer, PA1; and colon cancer, WiDr cells. However, no study has assessed the effect of a combination of 5-FU and CDDP in more than two cancer cell lines. Information on the cell line dependence of 5-FU and CDDP is, therefore, sparse. Furthermore, sequence dependence of these drugs has been shown, but schedule dependence, in terms of the timing of CDDP treatment, remains controversial. We therefore examined the cytotoxicity of 5-FU and CDDP in four different human gastric cancer cell lines by altering the times of CDDP treatment.

In our study, CDDP treatment 8 h or 16 h after the initial treatment with 5-FU was more cytotoxic than 5-FU after CDDP in three cell lines, excluding MKN-1. As for the timing of CDDP treatment, cytotoxic activity was greatest when CDDP was applied 8 h or 16 h after the start of treatment with 5-FU in MKN-28, MKN-45, and MKN-74 cells. These results support the idea of the

schedule dependence of the cytotoxic activity of 5-FU and CDDP. Against MKN-1 cells, cytotoxicity was greatest when CDDP was applied before 5-FU. These two conflicting results suggest that the cytotoxicity of 5-FU and CDDP is also cell-type-dependent, because MKN-1 is an adenosquamous cancer cell line, while the other three cell lines we used are derived from adenocarcinomas.

Our findings suggest that an optimal schedule for treatment with 5-FU and CDDP could be designed both by the timing of CDDP administration and according to the target cell type. Translating our in-vitro data into clinical practice might be difficult; however, a recent clinical trial with 5-FU continuous infusion plus CDDP bolus infusion for several consecutive days has provided a high response rate against gastric cancer [4,5]. In the regimen in this clinical trial, CDDP concentration is continuously maintained above the minimum effective concentration, resulting in sequence-independent broad cytotoxicity.

Conclusion

The cytotoxicity of a combination of 5-FU and CDDP is both schedule- and cell-line-dependent, and is especially affected by the timing of the CDDP treatment.

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