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ORIGINAL ARTICLE

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Intermittent FLDP: 24-h infusion of 5-FU on days 1, 3 and 5 combined with low-dose cisplatin on days 1–5 for gastric cancer, and its pharmacologic and kinetic rationale

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Abstract *Purpose*: To improve the therapeutic efficacy and minimize the toxicity of 5-fluorouracil (5-FU), intermittent therapy consisting of alternate 24-h intravenous infusion and based on differences in generation time (T_G) between normal cells and tumor cells was investigated. *Methods*: Two human gastric cancer cell lines MKN-7 and MKN-74 with T_G of 35 h and 17 h, respectively, were used in an in vitro cytotoxic assay. The drug exposure schedule consisted of a continuous 144-h exposure and alternate 24-h exposures. In a clinical trial, a total of 23 patients with advanced or recurrent gastric cancer were treated with intermittent therapy consisting of 24-h intravenous infusion with 5-FU 700 mg/m^2 per day on days 1, 3 and 5 in combination with low-dose cisplatin (CDDP) at 3.3 mg/m^2 per day on days 1 to 5. One cycle of the combined chemotherapy lasted for four consecutive weeks, followed by withdrawal over 1–2 weeks. Plasma 5-FU concentrations were measured by high-performance liquid chromatography in 15 patients and dihydropyrimidine dehydrogenase (DPD) activity in peripheral blood mononuclear cells (PBMC) was measured in 13 patients. *Results*: The in vitro study revealed no statistically significant difference in cytotoxicity of 5-FU between the two drug exposure schedules in MKN-7 cells. In MKN-74 cells, however, a statistically significant decrease in cytotoxicity was found with the alternate 24-h exposure. In a clinical trial, plasma 5-FU concentrations showed a trapezoidal pattern. There was a significant correlation between DPD activity in PBMC and total body clearance of 5-FU. There were eight partial responders (8/22, 36%). Toxicities were very mild in severity, with no grade 3 or 4 toxicity. In particular, diarrhea and stomatitis were infrequent (one patient), and none of the patients developed thrombocytopenia. *Conclusions*: Toxicities which may be observed in rapidly growing cells such as bone marrow cells and gastrointestinal epithelial cells following continuous intravenous infusion of 5-FU seemed to be reduced by intermittent therapy of 5-FU consisting of alternate 24-h intravenous infusions.

Keywords 5-Fluorouracil · Alternate 24-h intravenous infusion · Generation time · Dihydropyrimidine dehydrogenase

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Introduction

5-Fluorouracil (5-FU) was developed by Heidelberger et al. [9] more than four decades ago and is still in wide use as a chemotherapeutic agent, especially for gastrointestinal cancers. The mechanism of action of the drug is considered to differ depending on the regimen, i.e. disturbance of RNA function with bolus intravenous infusion and inhibition of DNA synthesis with continuous intravenous infusion [22]. Although several regimens have been studied against various human tumors, the optimal regimen for 5-FU is still a matter of debate. In recent studies in patients with colorectal cancer, continuous intravenous infusion has been shown to provide superior antitumor activity and induce less toxicity than intravenous bolus infusion. Myelosuppression is infrequent, and dose-limiting toxicities are hand-foot syndrome, stomatitis, and diarrhea with this regimen [6, 16, 18, 20].

Among several combination chemotherapies including 5-FU, the combination with cisplatin (CDDP) is considered to be one of the most successful for gastrointestinal cancers [15, 21, 23]. In Western countries, the standard protocol for CDDP infusion is a high-dose single short-term infusion [15, 23] or medium-dose daily short-term infusion [21]. In Japan, on the other hand, continuous intravenous infusion of 5-FU in combination with low-dose CDDP (continuous FLDP) has been extensively investigated, and the efficacy of the combination in the treatment of gastric and colorectal cancers has been demonstrated by several investigators [3, 13, 14, 32]. In this combination therapy, CDDP is used not as an effector but as a modulator of 5-FU [1, 25, 26, 27]. The toxicity of the combination therapy is generally minimal. However, myelosuppression, stomatitis and diarrhea after repeated cycles of continuous intravenous infusion of 5-FU are sometimes observed.

For the purpose of minimizing the toxicity of 5-FU while preserving its antitumor activity, we devised an intermittent 5-FU schedule which consisted of alternate 24-h infusions and is based on differences in generation time (T_G) between tumor cells and normal cells [28]. To elucidate the mechanism by which intermittent therapy reduces the toxicity of 5-FU, we used two gastric cancer cell lines with different T_G (17 h and 35 h) and investigated differences in cytotoxicity. We also investigated the clinical efficacy of low-dose CDDP consisting of alternate 24-h infusion of 5-FU in 23 patients with gastric cancer as well as the pharmacokinetic parameters and dihydropyrimidine dehydrogenase (DPD) activity in peripheral blood mononuclear cells (PBMC).

Materials and methods

Cells

Two human gastric cancer cell lines MKN-7 (T_G 35 h) and MKN-74 (T_G 17 h) were obtained from the Riken Cell Bank (Institute of Physical and Chemical Research, Saitama, Japan). These cells, cultured in RPMI-1640 medium containing 10% fetal calf serum (FCS; Gibco, Rockville, Md.), were incubated at 37°C in an atmosphere containing 5% CO_2. All experiments were conducted using cells in logarithmic growth phase.

Chemotherapeutic agent

5-FU was purchased from Kyowa Hakko Kogyo (Tokyo, Japan). The agent was dissolved into RPMI-1640 medium containing 10% FCS before use.

Colony-forming assay

The cytotoxicity of 5-FU was determined by the colony-forming assay. Cells were plated onto a 24-well tissue culture plate at 3×10^2 cells/ml per well for MKN-7 cells and 2×10^2 cells/ml per well for MKN-74 cells. Cells were precultured at 37°C in an atmosphere containing 5% CO_2 for 48 h. Various concentrations of 5-FU were added, and cells were incubated for a further 144 h. The cell treatment schedules consisted of a continuous 144-h exposure and alternate 24-h exposures. In the alternate 24-h exposure group, drug-containing medium was aspirated and washed twice with fresh medium. The medium was changed every 24 h in all wells to eliminate its effect on cell growth. After the 144-h incubation, the medium was removed and cells were fixed with 1 ml 10% formaldehyde solution for 30 min. Cells were then extensively washed with distilled water and stained with 0.1% crystal violet. Colonies were counted using an automatic colony analyzer.

Patients

Patients with histologically confirmed advanced gastric adenocarcinoma were eligible for the present study. All patients were required to meet the following eligibility criteria: <75 years of age, Eastern Cooperative Oncology Group performance status (PS) 0 to 3, no chemotherapy or radiotherapy during the 4 weeks prior to entry, normal bone marrow function (WBC $>4000/mm^3$ and platelets $>100{,}000/mm^3$), normal liver function (serum aspartate transaminase and serum alkaline phosphatase less than three times the upper limit of normal, and serum total bilirubin <1.5 mg/dl) and normal renal function (serum creatinine <1.2 mg/dl and creatinine clearance >60 ml/min). Informed consent was obtained from all patients prior to enrollment. The present study was conducted with the approval by the Institutional Review Board.

Treatment schedule

The regimen was as follows: 5-FU was continuously infused for 24 h at a dose of 700 mg/m^2 per day on days 1, 3 and 5 in combination with CDDP diluted in 100 ml 0.9% sodium chloride solution which was infused over 1 h at a dose of 3.3 mg/m^2 per day on days 1 to 5. One cycle lasted for four consecutive weeks, followed by withdrawal over 1 to 2 weeks. The combined chemotherapy was continued until the disease progressed or unacceptable toxicities developed.

Pharmacokinetic analysis of 5-FU

In 15 of 23 patients, plasma 5-FU concentrations were measured on day 1 of treatment. Plasma was collected at five time-points: just before 5-FU infusion, at 2, 12 and 24 h after the initiation of 5-FU infusion and 2 h after completion of 5-FU infusion. Plasma samples were stored at −70°C until assay. Plasma 5-FU concentrations were measured by high-performance liquid chromatography as previously described [19]. From plasma 5-FU concentration, steady-state concentration (C_{ss}), area under the curve (AUC) and total body clearance (Cl_{tot}) were calculated.

Measurement of DPD activity in PBMC

In 13 of 23 patients, DPD activity in PBMC was measured. Approximately 10 ml peripheral venous blood was collected at 8 a.m. from all patients to minimize circadian variability. PBMC were collected by differential centrifugation using Ficoll-Hypaque solution. DPD activity in PBMC was measured using a radioenzymatic method as described previously [31].

Assessment of response and toxicity

Patients were evaluated for response every 4 weeks by standard chest radiography, computed tomography, ultrasonography, and/or upper gastrointestinal radiography. Objective responses were classified according to the Japanese Classification of Gastric Carcinoma [10] as follows: complete response (CR), partial response (PR), no change (NC), and progressive disease (PD). Toxicities of

the regimen in each cycle were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2. The time to progression (TTP) was calculated from the start of treatment to disease progression, and survival was calculated from the start of treatment to death.

Statistical analysis

Statistical analysis was carried out with Stat View version 5.0 software (SAS Institute, Cary, N.C.). The significance of differences between two group was evaluated using to the Mann-Whitney *U*-test. To determine the degree of correlation between two variables, linear regression analysis was performed to calculate Spearman's rank correlation coefficient. Fischer's exact probability test was used for testing the correlation between DPD activity in PBMC and toxicity of 5-FU. Overall survival and TTP were calculated by the Kaplan-Meier method. *P*-values less than 0.05 were considered statistically significant.

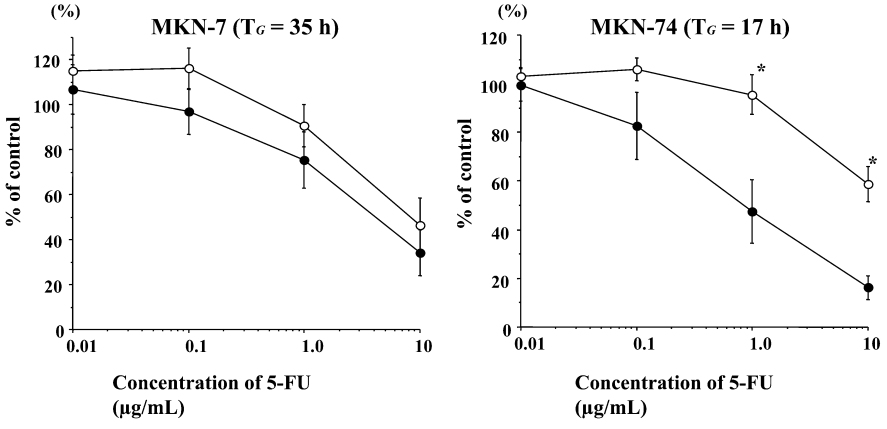
Results

Cytotoxicity of 5-FU determined by the colony-forming assay with different cell treatment schedules

Dose-response curves in MKN-7 cells (T_G 35 h) and MKN-74 cells (T_G 17 h) with different treatment schedules are shown in Fig. 1. MKN-7 cells showed no statistically significant decrease in cytotoxicity of 5-FU with either the continuous 144-h exposure or the alternate 24-h exposure. However, in MKN-74 cells, the cytotoxicity was significantly lower with the alternate 24-h exposure than with the continuous exposure. The 50% growth inhibitory concentrations (IC_{50}) calculated from the dose-response curves are shown in Table 1. In MKN-7 cells, the IC_{50} was about twofold higher with the alternate 24-h exposure than with the continuous 144-h exposure; however, the difference was not statistically significant. In MKN-74 cells, in contrast, the IC_{50} was 11-fold higher with the alternate 24-h exposure than with the continuous 144-h exposure; this difference was statistically significant.

To exclude differences in total drug exposure time, the product of the IC_{50} and total drug exposure time (C×T) was calculated and compared for each cell treatment schedule (Table 1). In MKN-7 cells, no significant difference was found in C×T between cell treatment schedules. However, the C×T was statistically significantly higher with the alternate 24-h exposure than with the continuous 144-h exposure in MKN-74 cells.

Fig. 1 Cytotoxicity of 5-FU determined by a colony-forming assay in relation to different cell treatment schedules. Cells were plated onto a 24-well culture plate. Cells precultured for 48 h were incubated with various concentrations of 5-FU for a further 144 h. The cell treatment schedules consisted of continuous (●) and alternate 24-hr (○) exposures to 5-FU. Cell viability was determined by counting the number of colonies. The mean numbers of colonies in the treated cultures were calculated as a percentage of the number in nontreated cultures. *Error bars* indicate standard error (*$P < 0.05$)



Patient characteristics

Enrolled in the present study were 23 patients of whom 16 had advanced gastric adenocarcinoma and 7 had recurrent gastric adenocarcinoma. One patient had an unmeasurable lesion. Therefore, response rates were calculated in 22 patients, and toxicity and survival were evaluated in 23 patients. Patient characteristics are summarized in Table 2. The median age of the patients

Table 1 Comparison of cytotoxicity between continuous exposure and alternate 24-hr exposure to 5-FU (T_G generation time)

Cell line	IC_{50} (μg/ml)			IC_{50} (μg h/ml) × exposure time		
	Continuous	Alternate 24-h	*P* value[a]	Continuous	Alternate 24-h	*P* value[a]
MKN-7 (T_G 35 h)	6.2 + 7.3	12.9 + 15.5	0.1489	886 + 1051	928 + 1117	0.7728
MKN-74 (T_G 17 h)	1.7 + 1.5	19.6 + 14.8	0.0090	242 + 208	1408 + 1065	0.0283

[a]Mann-Whitney *U*-test

Table 2 Patient characteristics

Age (years)	
Median	58.9
Range	34–76
Gender	
Male	12
Female	11
Performance status	
0	6
1	13
2	4
Histology	
Differentiated	5
Undifferentiated	16
Not determined	2
Prior chemotherapy	
None	16
Methotrexate/5-FU	3
Doxifluridine	2
Tegafur plus uracil (UFT)	1
Capecitabine	1

was 58.9 years (range 34 to 76 years). Of the 23 patients, 19 (83%) had good PS (0 or 1), 16 (70%) had undifferentiated gastric adenocarcinoma, and 7 (30%) had received 5-FU previously.

Response to treatment

Tumor responses to treatment are summarized in Table 3. There was no CR. PR was observed in eight patients, with an overall response rate of 36% (95% CI 15–58%). Response rates by site of tumor were 25%, 57%, 38%, 0% and 0% for the stomach, lymph nodes, peritoneum, liver and bone, respectively. No statistically significant difference was found in response rate according to histological type (differentiated 20% vs undifferentiated 44%) or prior treatment (none 31% vs prior fluoropyrimidine 43%). There was also no significant difference in response rate between patients with recurrent disease (29%) and patients with advanced disease (38%). The median TTP was 4.3 months, and the median survival was 6.5 months. One- and two-year survival rates were 18.2% and 6.1%, respectively.

Toxicities

Major toxicities are listed in Table 4. Toxicities were minimal, and no grade 3 or 4 toxicity was observed. The most frequently observed toxicities were nausea, leukocytopenia, neutropenia and anemia, which developed in about half of the patients. Hand-foot syndrome was observed in two patients who received two or more cycles of the present regimen. Diarrhea and stomatitis were infrequent (one patient each), and none of the patients developed thrombocytopenia.

Pharmacology of 5-FU

Changes in plasma 5-FU concentration are shown in Fig. 2. Plasma 5-FU concentrations increased soon after the infusion of the drug and remained at a plateau level during infusion. After termination of infusion, plasma 5-FU concentrations rapidly decreased to almost zero. The C_{ss} and AUC were 226.2 ± 72.9 ng/ml (range 112–387 ng/ml, coefficient of variability, CV, 32.2%) and 5924 ± 2575 ng h/ml (range 2,575–11,595 ng h/ml, CV 43.5%), respectively. Similarly, the Cl_{tot} of 5-FU was 231.4 ± 98.8 l/h per m^2 (range 118–436 l/h per m^2, CV 42.7%). None of these pharmacokinetic parameters was correlated with therapeutic effect or toxicities (data not shown).

DPD activity in PBMC

DPD activity in PBMC ranged from 69 to 480 pmol/mg per min (median 217 pmol/mg per min). There was a significant linear relationship between DPD activity in PBMC and Cl_{tot} of 5-FU ($r_s = 0.674$, $P = 0.0195$; Fig. 3). There was no correlation between DPD activity in PBMC and therapeutic effect. However, if the cut-off level of DPD activity in PBMC was set at 175 pmol/mg per min, the incidence of leukocytopenia and neutropenia was significantly higher among patients with low DPD activity than among those with high DPD activity (Table 5).

Discussion

Recently, the efficacy of FLDP therapy against gastrointestinal cancers has been extensively investigated in Japan [3, 13, 14, 32]. The regimen currently used is CDDP 5–10 mg and 5-FU 330–500 mg per patient per day infused intravenously 5 days per week. The rates of efficacy with this regimen have been reported to be 45 to

Table 3 Response to treatment

	No. of patients	CR	PR	NC	PD	Response rate (%)
Overall	22	0	8	11	3	36
Site of tumor						
Stomach	13	0	4	9	0	31
Lymph nodes	7	0	4	3	0	57
Peritoneum	8	0	3	5	0	38
Liver	3	0	0	2	1	0
Bone	3	0	0	2	1	0

Table 4 Major toxicities (evaluated according to NCI-CTC version 2)

Toxicity	Grade				
	0	1	2	3	4
Nausea	11	8	4	0	0
Diarrhea	22	1	0	0	0
Stomatitis	22	1	0	0	0
Hand-foot syndrome	21	0	2	0	0
Leukocytopenia	12	8	3	0	0
Neutropenia	13	7	3	0	0
Thrombocytopenia	23	0	0	0	0
Anemia	15	5	3	0	0

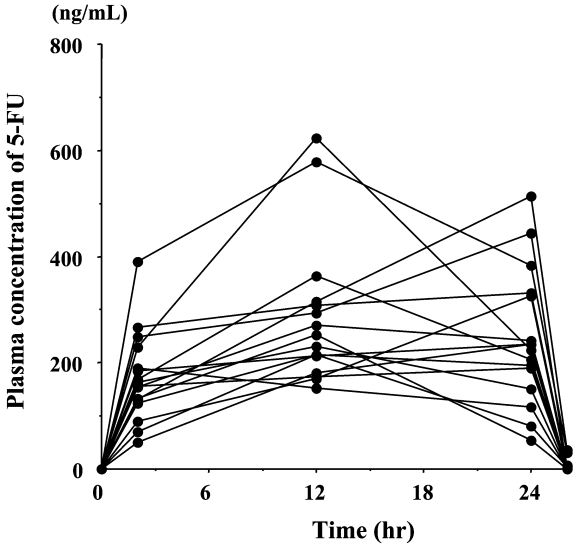


Fig. 2 Pharmacokinetic profile of 5-FU in human plasma. 5-FU was infused intravenously for 24 h at a dose of 700 mg/m^2 in patients with histologically confirmed advanced gastric adenocarcinoma. Plasma samples were collected immediately before infusion, 2, 12 and 24 h after infusion and 2 h after the completion of infusion. Plasma 5-FU concentrations were determined by high-performance liquid chromatography

50% for gastric cancer. The mechanism of action of this combination chemotherapy is considered to involve the biochemical modulation of 5-FU by CDDP, i.e. CDDP is used not as an effector but as a modulator of 5-FU as described previously [1, 25, 26, 27]. Life-threatening toxicities have not been observed with this combination chemotherapy [3, 13, 14]. However, thrombocytopenia, leukopenia and diarrhea are considered to be dose-limiting toxicities during repeated cycles of this therapy [28]. Therefore, we devised intermittent therapy of 5-FU to reduce its toxicity based on differences in T_G between tumor cells and normal cells.

Lipkin et al. [17] and Clarkson et al. [4] investigated T_G of human normal cells and tumor cells using the ^{3}H-dThd labeling regimen. They reported that T_G in tumor cells are generally 5–7 days and T_G in normal cells, e.g. bone marrow cells and intestinal epithelium, are 0.5–2 days. Their findings support the evidence that

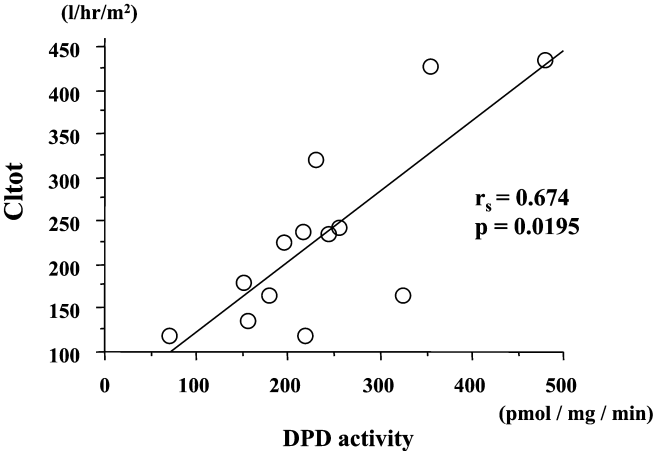


Fig. 3 Correlation between DPD activity in PBMC and total body clearance of 5-FU (*Cltot*). A significant positive correlation was observed between DPD activity in PBMC and body clearance of 5-FU ($r_s = 0.674$, $P = 0.0195$)

Table 5 Toxicities (evaluated according to NCI-CTC version 2) in relation to DPD activity in PBMC

Toxicity	Incidence of toxicity		P value[a]
	Low DPD	High DPD	
Nausea	2/3	6/10	0.8351
Diarrhea	1/3	0/10	0.2308
Stomatitis	1/3	0/10	0.2308
Hand-foot syndrome	1/3	0/10	0.2308
Leukocytopenia	3/3	2/10	0.0350
Neutropenia	3/3	2/10	0.0350
Anemia	1/3	3/10	0.6307

[a]Fisher's exact probability test

toxicities against normal cells with shorter T_G than tumor cells, e.g. bone marrow cells and intestinal epithelial cells, are dose-limiting with long-term continuous infusion of S-phase-specific agents as represented by 5-FU. Based on this valuable discovery, we used two gastric cancer cell lines, i.e. MKN-7 with a long T_G (35 h) and MKN-74 with a short T_G (17 h), as a cancer cell model and a normal cell model, respectively, in an attempt to determine whether differences in T_G might be associated with a reduction in 5-FU's cytotoxicity while preserving its antitumor activity. MKN-7 cells showed no statistically significant decrease in cytotoxicity with either the continuous 144-h exposure or the alternate 24-h exposure, in contrast to MKN-74 cells which showed statistically significantly reduced cytotoxicity with the alternate 24-h exposure compared to continuous exposure.

Saga et al. [24] also investigated differences in cytotoxicity between 96-h continuous exposure and alternate 6- and 24-h exposures in ovarian cancer cell lines with short T_G (15 h) and long T_G (45 h). They found a statistically significant decrease in cytotoxicity with the alternate 24-h exposure in the cell line with a shorter T_G than the drug exposure interval, and found that the

difference in cytotoxicity was minimal in the cell line with a longer T_G than the drug exposure interval. Therefore, we hypothesized that intermittent 5-FU therapy with a drug exposure interval longer than the T_G might reduce its cytotoxicity. Since the duration of S-phase has been reported to be about 10 h in normal cells and 17–60 h in tumor cells [4], intermittent 5-FU therapy with a drug exposure interval of not shorter than 10 h and not longer than 60 h may sufficiently preserve antitumor activity with less cytotoxicity as compared with continuous FLDP therapy.

This hypothesis was confirmed in a clinical trial. The overall response rate was 36% with intermittent FLDP therapy. Response rates with continuous FLDP therapy in a phase II study carried out at a different institute have been reported to be from 45 to 50% [3, 13, 14]. Recently, Toge et al. [32] reviewed FLDP therapy at 82 medical institutes in Japan and reported that the overall response rate was 35%. The above-mentioned reports allow us to conclude that the efficacy is almost comparable between intermittent FLDP therapy and continuous FLDP therapy.

A pharmacokinetic review has revealed differences in the time-point at which plasma 5-FU concentrations peak from one patient to another, which might be due to differences in each individual's circadian rhythm [7]. However, plasma 5-FU concentrations showed a trapezoidal pattern in most patients. The pharmacokinetic profile of 5-FU was quite similar between cell treatment in vitro and intermittent therapy in the clinical setting, providing coincidence between a preclinical study and a clinical trial. Continuous infusion of 5-FU at a dose of 300 mg/m^2 per day is a commonly used regimen in the treatment of colorectal cancer. Jodrell et al. [11] reported that the plasma C_{ss} of 5-FU was 94 ± 25 ng/ml in this dose setting and estimated the AUC per week as 15,792 ng h/ml. In the present study, the AUC of 5-FU per week, which was calculated to be 17,772 ng h/ml (AUC 5924 ng h/ml $\times 3$), was greater than the value with continuous infusion of 5-FU. Severe stomatitis and diarrhea have been reported with continuous intravenous infusion of 5-FU [6, 16, 18]. In the present clinical trial, on the other hand, stomatitis and diarrhea, if they occurred at all, were infrequent and mild despite combined use of CDDP. Although myelosuppression is not considered to be dose-limiting with continuous intravenous infusion of 5-FU, leukopenia seemed to be less severe in the present study than with continuous intravenous infusion of 5-FU and continuous FLDP therapy [3, 14, 16, 18]. These results strongly support the concept that toxicities in rapidly growing cells, especially gastrointestinal epithelial cells, are reducible by intermittent therapy of 5-FU.

Recently, it has been found that DPD, an initial and rate-limiting catabolic enzyme, has significance for the pharmacokinetics and toxicity of 5-FU [7, 8], and it has been reported that patients with DPD deficiency show severe toxicity to 5-FU administration [29]. It has also been reported that, in tumors with high DPD activity, 5-FU decomposition is accelerated, resulting in resistance to 5-FU [2, 31]. In the present study, we found a positive correlation between DPD activity in PBMC and Cl_{tot} of 5-FU. Fleming et al. have also demonstrated a significant relationship between DPD activity in PBMC and 5-FU systemic clearance [5]. Thus, it is probable that a relationship exists between DPD activity in PBMC and the systemic pharmacokinetics of 5-FU. Furthermore, we found a significant relationship between DPD activity in PBMC and toxicity of 5-FU in the present study. Katona et al. investigated the relationship between DPD activity in PBMC and the toxicity of 5-FU in 48 patients with colorectal cancer and found that the incidence of toxicity was significantly higher in patients with low DPD activity than in those with moderate or high DPD activity [12].

We have previously investigated the relationship between DPD activity in PBMC and the toxicity of oral fluoropyrimidines and have found that DPD activity in PBMC is significantly lower in patients showing toxicity to oral fluoropyrimidines than in those without toxicity. In addition, the toxicity of oral fluoropyrimidines can be predicted if the cut-off level of DPD activity is set at 175 pmol/mg per min [30]. Thus, we used the same cut-off level of DPD activity in the present study. These findings indicate that monitoring of DPD activity in PBMC is useful as a marker of 5-FU toxicity. However, as the sample size was too small to draw a definite conclusion, a further prospective trial should be carried out to confirm the relationship between DPD activity in PBMC and 5-FU toxicity.

In the present study, intermittent therapy with 5-FU with a drug exposure interval longer than the T_G showed a marked decrease in cytotoxicity while sufficiently preserving antitumor activity compared to continuous intravenous infusion of 5-FU. Our therapeutic approach provides 5-FU-based chemotherapy based on the novel concept of utilizing differences in T_G between tumor cells and normal cells. Although this concept seems to be applicable to other antimetabolites which exert cytotoxicity in an S-phase-specific manner, no definitive conclusion can be drawn because of the small sample size in the present clinical trial. To verify the clinical applicability of this concept, a large-scale, randomized, controlled clinical trial to compare continuous FLDP therapy and intermittent FLDP therapy should be conducted in the future.

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References

1. Araki H, Fukushima M, Kamiyama Y, Shirasaka T (2000) Effect of consecutive lower-dose cisplatin in enhancement of 5-fluorouracil cytotoxicity in experimental tumor cells in vivo. Cancer Lett 160:185

2. Beck A, Etienne MC, Cheradame S, Fischel JL, Formento P, Renee N, Milano G (1994) A role for dihydropyrimidine dehydrogenase and thymidylate synthase in tumour sensitivity to fluorouracil. Eur J Cancer 30A:1517
3. Chung YS, Yamashita Y, Inoue T, Matsuoka T, Nakata B, Onoda N, Maeda K, Sawada T, Kato Y, Shirasaka T, Sowa M (1997) Continuous infusion of 5-fluorouracil and low dose cisplatin infusion for the treatment of advanced and recurrent gastric adenocarcinoma. Cancer 80:1
4. Clarkson B, Ota K, Ohkita T, O'Connor A (1965) Kinetics of proliferation of cancer cells in neoplastic effusions in man. Cancer 18:1189
5. Fleming RA, Milano G, Thyss A, Etienne MC, Renee N, Schneider M, Demard F (1992) Correlation between dihydropyrimidine dehydrogenase activity in peripheral mononuclear cells and systemic clearance of fluorouracil in cancer patients. Cancer Res 52:2899
6. Hansen RM, Ryan L, Anderson T, Krzywda B, Quebbeman E, Benson A, Haller DG, Tormey DC (1996) Phase III study of bolus versus infusion fluorouracil with or without cisplatin in advanced colorectal cancer. J Natl Cancer Inst 88:668
7. Harris BE, Song R, Soong SJ, Diasio RB (1990) Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. Cancer Res 50:197
8. Heggie GD, Sommadossi JP, Cross DS, Huster WJ, Diasio RB (1987) Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. Cancer Res 47:2203
9. Heidelberger C, Chaudhuri NK, Dannenberg P, Mooren D, Griesbach L, Duschinsky R, Schnitzer RJ, Pleven E, Scheiner J (1957) Fluorinated pyrimidines, a new class of tumor-inhibitory compounds. Nature 179:663
10. Japanese Gastric Cancer Association (2001) Japanese classification of gastric carcinoma—2nd English edition—response assessment of chemotherapy and radiotherapy for gastric carcinoma: clinical criteria. Gastric Cancer 4:1
11. Jodrell DI, Stewart M, Aird R, Knowles G, Bowman A, Wall L, McLean C (2001) 5-Fluorouracil steady state pharmacokinetics and outcome in patients receiving protracted venous infusion for advanced colorectal cancer. Br J Cancer 84:600
12. Katona C, Kralovanszky J, Rosta A, Pandi E, Fonyad G, Toth K, Jeney A (1998) Putative role of dihydropyrimidine dehydrogenase in the toxic side effect of 5-fluorouracil in colorectal cancer patients. Oncology 55:468
13. Kim R, Murakami S, Ohi Y, Inoue H, Yoshida K, Toge T (1999) A phase II trial of low dose administration of 5-fluorouracil and cisplatin in patients with advanced and recurrent gastric cancer. Int J Oncol 15:921
14. Kondo K, Murase M, Kodera Y, Akiyama S, Ito K, Yokoyama Y, Takagi H, Shirasaka T (1996) Feasibility study on protracted infusional 5-fluorouracil and consecutive low-dose cisplatin for advanced gastric cancer. Oncology 53:64
15. Lacave AJ, Baron FJ, Anton LM, Estrada E, De Sande LM, Palacio I, Esteban E, Gracia JM, Buesa JM, Fernandez OA, et al (1991) Combination chemotherapy with cisplatin and 5-fluorouracil 5-day infusion in the therapy of advanced gastric cancer: a phase II trial. Ann Oncol 2:751
16. Leichman CG, Fleming TR, Muggia FM, Tangen CM, Ardalan B, Doroshow JH, Meyers FJ, Holcombe RF, Weiss GR, Mangalik A (1995) Phase II study of fluorouracil and its modulation in advanced colorectal cancer: a Southwest Oncology Group study. J Clin Oncol 13:1303
17. Lipkin M, Sherlock P, Bell B (1963) Cell proliferation kinetics in the gastrointestinal tract of man. J Clin Invest 45:721
18. Lokich JJ, Ahlgren JD, Gullo JJ, Philips JA, Fryer JG (1989) A prospective randomized comparison of continuous infusion fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma: a Mid-Atlantic Oncology Program study. J Clin Oncol 7:425
19. Masuike T, Watanabe I, Takemoto Y (1985) Quantitative method of 5-fluorouracil and its metabolites in biological samples using high performance liquid chromatography (in Japanese). Yakugaku Zasshi 105:1058
20. Meta-Analysis Group in Cancer (1998) Toxicity of fluorouracil in patients with advanced colorectal cancer: effect of administration schedule and prognostic factors. J Clin Oncol 16:3537
21. Ohtsu A, Shimada Y, Yoshida S, Saito H, Seki S, Morise K, Kurihara M (1994) Phase II study of protracted infusional 5-fluorouracil combined with cisplatinum for advanced gastric cancer: report from the Japan Clinical Oncology Group (JCOG). Eur J Cancer 30A:2091
22. Parker WB, Cheng YC (1990) Metabolism and mechanism of action of 5-fluorouracil. Pharmacol Ther 48:381
23. Rougier P, Ducreux M, Mahjoubi M, Pignon JP, Bellefqih S, Oliveira J, Bognel C, Lasser P, Ychou M, Elias D, et al (1994) Efficacy of combined 5-fluorouracil and cisplatinum in advanced gastric carcinomas. A phase II trial with prognostic factor analysis. Eur J Cancer 30A:1263
24. Saga Y, Suzuki M, Sato I, Shirasaka T (2000) An in vitro examination of a 5-fluorouracil regimen involving continuous venous infusion using cultured cell lines derived from ovarian cancers. Oncol Rep 7:625
25. Scanlon KJ, Safirstein RL, Thies H, Gross RB, Waxman S, Guttenplan JB (1983) Inhibition of amino acid transport by cis-diamminedichloroplatinum(II) derivatives in L1210 murine leukemia cells. Cancer Res 43:4211
26. Scanlon KJ, Newman EM, Lu Y, Priest DG (1986) Biochemical basis for cisplatin and 5-fluorouracil synergism in human ovarian carcinoma cells. Proc Natl Acad Sci U S A 83:8923
27. Shirasaka T, Aiba K, Araki H, Suzuki M, Terashima M, Mikami Y (1999) Combination therapy of continuous venous infusion (CVI) of 5-FU and low dose consecutive cisplatin (CDDP), and the new oral anti-cancer drug S-1 for advanced gastro-intestinal cancer. Jpn J Cancer Chemother 26:456
28. Shirasaka T, Yamamitsu S, Tsuji A, Taguchi T (2000) Conceptual changes in cancer chemotherapy: from an oral fluoropyrimidine prodrug, UFT, to a novel oral fluoropyrimidine prodrug, S-1, and low-dose FP therapy in Japan. Invest New Drugs 18:315
29. Takimoto CH, Lu ZH, Zhang R, Liang MD, Larson LV, Cantilena LR Jr, Grem JL, Allegra CJ, Diasio RB, Chu E (1996) Severe neurotoxicity following 5-fluorouracil-based chemotherapy in a patient with dihydropyrimidine dehydrogenase deficiency. Clin Cancer Res 2:477
30. Terashima M, Fujiwara H, Abe K, Sasaki N, Tkagane A, Oyama K, Saito K (2002) Toxicity of oral fluoropyrimidines is predictable by dihydropyrimidine dehydrogenase activity in peripheral blood mononuclear cell (abstract 617). Proc Am Soc Clin Oncol 21:155a
31. Terashima M, Irinoda T, Fujiwara H, Nakaya T, Takagane A, Abe K, Yonezawa H, Oyama K, Inaba T, Saito K, Takechi T, Fukushima M (2002) Role of thymidylate synthase and dihydropyrimidine dehydrogenase on tumor progression and sensitivity to 5-fluorouracil in human gastric cancer. Anticancer Res 22:761
32. Toge T, Nakazato H, Nishiyama M, Hirata K, Yamamitsu S, Sowa M, Saji S (2000) Current status of "low-dose cisplatin-5-FU therapy" for solid tumors (2nd report)—from a nationwide questionnaire on its adverse effects (in Japanese). Jpn J Cancer Chemother 27:549