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ORIGINAL ARTICLE

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Thymidylate synthase inhibition by an oral regimen consisting of tegafur-uracil (UFT) and low-dose leucovorin for patients with gastric cancer

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Abstract *Purpose*: 5-Fluorouracil (5-FU) remains a standard therapy for patients with advanced gastric cancer. There has been no study using an oral regimen with a combination of tegafur, a masked compound of 5-FU, and leucovorin in gastric cancer. The purpose of this study was to determine whether orally administered low-dose leucovorin enhances thymidylate synthase (TS) inhibition when added to tegafur-uracil (UFT) in patients with gastric cancer. *Methods*: A group of 26 patients with resectable gastric cancer were assigned to one of two regimens: UFT alone or UFT plus leucovorin. UFT, equivalent to 400 mg/day tegafur, with or without 30 mg/day leucovorin, was administered orally in divided daily doses every 12 h for 3 consecutive days prior to surgery. Tumor specimens were taken immediately following gastrectomy, and the TS inhibition rate (TSIR) was determined using a ligand-binding assay. *Results*: The TSIR was significantly higher in the UFT plus leucovorin group than in the UFT alone group ($P < 0.01$). The TSIR in the patients treated with UFT alone ranged between 14% and 50%, while six of the eight patients treated with UFT plus leucovorin had a TSIR of 55% or higher. The remaining two patients in the group treated with UFT plus leucovorin, with a TSIR of 31% and 44%, had undifferentiated tumors. *Conclusion*: Our results suggest that orally administered low-dose leucovorin can add to the efficacy of UFT in patients with gastric cancer, and provide preliminary data for a randomized clinical trial.

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Introduction

Although the incidence of gastric cancer has decreased yearly worldwide, it remains a major cause of death in Japan and in some other countries. 5-Fluorouracil (5-FU), as a single agent or in combination with other drugs, has been a standard therapy for patients with metastatic or recurrent gastric cancer. 5-FU is metabolized to 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), which binds to and inhibits thymidylate synthase (TS) and blocks DNA synthesis. This is considered to be the dominant mechanism of its action at clinical doses, although interference with RNA function may be another [4, 5, 12]. The degree of TS inhibition in tumor tissue has been found to be an important indicator for the efficacy of 5-FU or its derivatives [9, 12, 21–27]

Prospective randomized studies have revealed that leucovorin, D, L-5-formyltetrahydrofolate, adds to the efficacy of 5-FU in patients with colorectal cancer [2, 3, 13, 17, 18]. Following administration, L-5-formyltetrahydrofolate is metabolized to 5,10-methylenetetrahydrofolate, which binds to the FdUMP/TS complex, forming a more stable ternary complex and enhancing the effect of 5-FU. In gastric cancer, a few studies have reported the possible value of leucovorin when added to 5-FU [1, 7, 10, 11], although there has been no randomized study comparing the two regimens of 5-FU or its derivatives with and without leucovorin.

UFT (Taiho Pharmaceutical Co., Tokyo) is a mixture of tegafur, 1-(2-tetrahydrofuryl)-5-fluorouracil, and uracil at a molar ratio of 1:4. Orally administered tegafur is metabolized to 5-FU in the liver, and uracil can potentiate the effect of 5-FU through interference with its catabolism. Oral administration of drugs may

be preferred by patients to intravenous administration. There has been no study using an oral regimen of UFT and leucovorin in patients with gastric cancer. The purpose of this study was to determine whether or not orally administered low-dose leucovorin enhances TS inhibition when combined with UFT in patients with gastric cancer.

Patients and methods

From October 1992 to June 1994, 26 patients with resectable adenocarcinoma of the stomach at the First Department of Surgery, National Defense Medical College Hospital were enrolled in this study. Patients were required to have a leukocyte count of greater than 3000/mm^3, a platelet count of greater than 150 000/mm^3 and a serum creatinine concentration of less than 1.5 mg/dl. They were randomly assigned to one of two regimens: UFT alone or UFT plus leucovorin. Four capsules per day of UFT, equivalent to 400 mg/day tegafur, with or without 30 mg/day leucovorin, were administered orally for 3 consecutive days immediately prior to surgery. Half of the total daily dose of the drugs was administered every 12 h concurrently. The time of the last administration was set at approximately 6 h prior to gastric resection because the TS inhibition rate has been reported to remain maximal for approximately 2 to 8 h following oral administration of UFT in an experimental model [25].

Specimens weighing approximately 0.5 g were taken from the edge of the tumor immediately following gastrectomy and maintained at -70°C until the TS assay. We determined the activity of total TS and non-FdUMP-bound TS (free TS) using the assay of Spears et al. [22, 23]. Briefly, the specimens were thawed at 4 °C, homogenized by sonication, and centrifuged at 16 000 *g* for 3 h at 4°C. Aliquots (50 µl) of the supernatant were diluted with 50 µl buffer, pH 8.1, consisting of 0.3 M NH_4HCO_3, 100 m*M* mercaptoethanol, 100 m*M* NaF and 15 m*M* cytidine monophosphate, and incubated at 25 °C for 3 h to allow the FdUMP-bound TS to dissociate. Samples were then incubated with 6 p*M* [^{3}H]FdUMP (23 Ci/m*M*) in 50 µl 5 m*M* potassium phosphate buffer, pH 7.4, and 25 µl 50 m*M* potassium phosphate buffer, pH 7.4, containing 2 m*M* tetrahydrofolate (FH_4), 20 m*M* mercaptoethanol, 100 m*M* NaF, 15 m*M* cytidine monophosphate, 2% bovine serum albumin, 16 m*M* sodium ascorbate and 9 m*M* formaldehyde at 25°C for 20 min. Protein-bound [^{3}H]FdUMP was isolated by the addition of dextran-coated charcoal and counted for radioactivity following centrifugation at 2 200 *g* for 30 min at 4°C. Potassium phosphate buffer, pH 7.4, was used as a blank in place of the cytosol supernatant. When the activity of free TS was determined, the samples were maintained at 0°C for 3 h prior to incubation with [^{3}H]FdUMP and FH_4 to prevent the dissociation of the FdUMP-bound TS. The free TS values were corrected for the dissociation which occurs during the incubation with [^{3}H]FdUMP and FH_4 according to the formula used by Spears et al.:

$$TS_{free}[c] = (TS_{free}[e] - 0.13\ TS_{total})/0.87$$

where $TS_{free}[c]$ is the corrected free TS value, $TS_{free}[e]$ is the experimentally determined free TS value and TS_{total} is the total TS value [22]. The inhibition of TS is expressed as the TS inhibition rate (TSIR), which was defined as: $(TS_{total} - TS_{free}[c])/TS_{total} \times 100\,(\%)$.

The clinicopathologic findings were described according to the Japanese classification of gastric carcinoma proposed by the Japanese Research Society for Gastric Cancer [6]. The differences between the means of the continuous variables between the two groups were tested using Student's *t*-test, and the significance of the differences in the frequency distributions were determined using the chi-squared test. A *P*-value of less than 0.05 was considered statistically significant. The histologic effects of the two regimens were evaluated on hematoxylin-eosin stained sections and classified into grade 0 to 3 according to the Japanese classification of gastric carcinoma [6]. Informed consent was obtained from all patients.

Results

We could not determine the TS activity in 4 of the 13 patients receiving UFT alone and in 5 of the 13 patients receiving UFT plus leucovorin. One of the specimens was lost during preservation. We could not detect the activity of total TS or free TS in two patients. Five patients had no free TS activity detectable with a low total TS activity. Thus, the analyses were performed on the remaining 17 patients. The clinicopathologic data of the two groups of patients are shown in Table 1.

Table 1 Clinicopatologic factors. Numbers in parentheses are percentages

	UFT alone	UFT plus leucovorin
Number of patients	9	8
Age (mean ± SD, years)*	70.8 ± 7.0	62.1 ± 8.7
Sex (male/female)	6/3	4/4
Positive liver metastasis	1 (11)	1(13)
Positive peritoneal metastasis	2 (22)	–
Gross findings		
Type 1	–	2 (25)
Type 2	5 (56)	3 (38)
Type 3	–	2 (25)
Type 4	4 (44)	1 (13)
Diameter of tumor (mean ± SD, cm)	7.4 ± 4.1	5.5 ± 2.1
Depth of tumor invasion**		
Mucosa, submucosa	–	2 (25)
Muscularis propria, subserosa	3 (33)	5 (63)
Serosa, adjacent structure	6 (67)	1 (13)
Lymph node involvement		
n0	3 (33)	3 (38)
n1	3 (33)	2 (25)
n2	2 (22)	2 (25)
n3, n4	1 (11)	1 (13)
Histologic classification[a]		
Differentiated	3 (33)	4 (50)
Undifferentiated	6 (67)	4 (50)
Growth pattern		
Expanding	1 (11)	–
Intermediate	4 (44)	6 (75)
Infiltrative	4 (44)	2 (25)
Stroma in tumor		
Medullary	1 (11)	1 (14)
Intermediate	6 (67)	5 (71)
Scirrhous	2 (22)	1 (14)
Lymph vessel invasion		
ly0 (no invasion)	–	1 (13)
ly1 (minimal invasion)	3 (33)	4 (50)
ly2 (moderate invasion)	3 (33)	1 (13)
ly3 (marked invasion)	3 (33)	2 (25)
Venous invasion		
v0 (no invasion)	3 (33)	3 (38)
v1 (minimal invasion)	2 (22)	1 (13)
v2 (moderate invasion)	2 (22)	4 (50)
v3 (marked invasion)	2 (22)	–

[a] *Differentiated* papillary and tubular adenocarcinoma, *Undifferentiated* poorly differentiated and mucinous adenocarcinoma
*$P < 0.05$ by Student's *t*-test,** $P < 0.05$ by the Chi-squared test

Table 2 Thymidylate synthase assay (*TS* thymidylate synthase, *NS* not significant)

	UFT alone	UFT plus leucovorin	
Number of patients	9	8	
Total TS (pmol/g wt)	11.9 ± 15.4	16.6 ± 14.9	NS
Free TS (pmol/g wt)	9.1 ± 13.8	6.1 ± 6.0	NS
TS inhibition rate (%)	32.5 ± 13.9	61.1 ± 18.1	$P < 0.01$

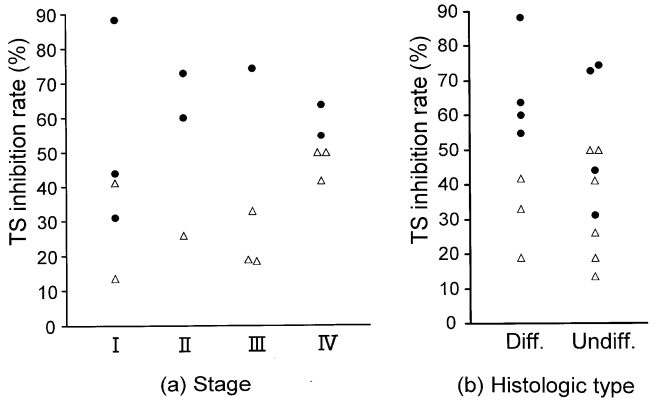

Fig. 1a, b Influence of the stage of the disease (a) and histologic type (b) on the TS inhibition rate (*open triangles* patients receiving UFT only, *circles* patients receiving UFT plus leucovorin) Stage of disease and histologic type were classified according to the Japanese classification of gastric carcinoma proposed by the Japanese Research Society for Gastric *Cancer* (*Diff.* papillary or tubular adenocarcinoma, *Undiff.* poorly differentiated or mucinous adenocarcinoma)

The average age appeared to be higher and the depth of tumor invasion deeper in the patients receiving UFT alone. However, there were no significant differences in any of the other factors between the two groups.

No significant differences were observed in the activity of either total TS or free TS between the two treatment groups. The TSIR, however, was significantly higher in the group receiving UFT plus leucovorin than in the group receiving UFT alone ($P < 0.01$; Table 2). The TSIR in the patients treated with UFT alone ranged between 14% and 50%, while six of the eight patients treated with UFT plus leucovorin had a TSIR of 55% or higher. The remaining two patients in the group treated with UFT plus leucovorin, with a TSIR of 31% and 44%, had undifferentiated tumors. However, there was no significant difference in the average TSIR between those with differentiated tumors and those with undifferentiated tumors in either treatment group. The stage of the disease did not influence the TSIR in either group (Fig. 1).

A histologic response of grade 2 or greater was not observed in any of the tumors, and there was no difference between the two treatment groups. No patient suffered any notable toxicity such as bone marrow suppression, oral pain or ulceration, nausea, vomiting or diarrhea.

Discussion

Our study revealed that the group receiving UFT plus leucovorin had a significantly higher TSIR than the group receiving UFT alone. The TSIR is considered to be an important indicator for the antitumor effect of 5-FU and its derivatives. The degree of TS inhibition in the tumor tissue has been reported to correlate with the antitumor activity of 5-FU or its derivatives in experimental tumor models or human tumor xenografts in nude mice [9, 12, 22, 25]. TS inhibition has been reported to be associated with a clinical and histologic response to 5-FU treatment [21, 23, 24, 26, 27]. Therefore, our results suggest that 30 mg/day of orally administered leucovorin can add to the efficacy of 400 mg/day UFT in patients with gastric cancer. The optimum dose of UFT is 300–600 mg/day as tegafur, and the maximum tolerated dose is 750 mg/day. The administration of over 400 mg/day UFT might be dangerous just before surgery. The small size of nine tumor specimens may have prevented us from determining the TS activity in samples from these patients. Peters et al. have also reported that TS was not detectable in a number of patients using the ligand-binding assay [16].

The combination of 5-FU and leucovorin has been reported to be associated with a higher response rate and better survival compared with 5-FU alone in prospective randomized studies in patients with colorectal cancer [2, 3, 13, 17, 18]. In gastric cancer, some studies have shown a response rate of 40% or higher with a combination of 5-FU and either low-dose or high-dose leucovorin [7, 10, 11], while a lower response rate has been reported in other studies [1].

Petrelli et al. have reported a trend toward a higher response rate and a longer survival with a 5-FU plus high-dose leucovorin regimen [17]. However, most studies have found no therapeutic advantage associated with the use of high-dose leucovorin compared with low-dose leucovorin when added to 5-FU in colorectal cancer [13, 18]. Okabe et al. have reported that 20–500 mg/m^2 leucovorin potentiates the cytotoxicity of UFT independent of the dose in an experimental model [14]. Therefore, we chose a low dose for leucovorin.

It has been reported that histologic degeneration occurs in patients with a TSIR of more than 50% after 5-FU or tegafur is given for 7 to 14 days [21, 26]. We did not observe any definite histologic effect with either of the two regimens even though six of the eight patients receiving UFT plus leucovorin had a TSIR of 55% or higher, probably because the period of administration was too short.

Oral regimens require patients to go to a hospital or clinic less frequently than intravenous regimens of 5-FU plus leucovorin in which the drugs are given 5 days a week, leading to a better quality of life and a lower

financial cost. Orally administered leucovorin has been reported to enhance the antitumor effect of UFT in experimental models [14]. It has been found that an oral regimen combining UFT with leucovorin results in a response rate of 25% to 42% in patients with colorectal cancer [15, 19, 20]. However, there has been no study using an oral regimen of UFT and leucovorin in patients with gastric cancer. Our study may provide preliminary data for a randomized clinical trial to define the therapeutic advantage of UFT plus leucovorin over UFT alone in gastric cancer. The combination of UFT and low-dose leucovorin on an outpatient basis may be a useful regimen, particularly as adjuvant therapy following surgical resection.

The regimen of sequential methotrexate and 5-FU has been reported to be more effective in poorly differentiated carcinomas of the stomach than in well-differentiated tumors [8]. There seemed to be a distinct difference in the TSIR between the regimens of UFT alone and UFT plus leucovorin in the differentiated tumors although we were unable to obtain conclusive evidence concerning the relationship between the histologic type and the efficacy of UFT plus leucovorin. It would be helpful if one could choose either of these two ways of biochemical modulation according to the histologic type of the tumor.

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