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The ratio of thymidine phosphorylase to dihydropyrimidine dehydrogenase in tumour tissues of patients with metastatic gastric cancer is predictive of the clinical response to 5′-deoxy-5-fluorouridine

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Abstract

The aim of this work was to determine whether intratumour contents of thymidine phosphorylase (TP), which converts 5′-deoxy-5-fluorouridine (5′-DFUR) to 5-fluorouracil, and dihydropyrimidine dehydrogenase (DPD), which degrades 5-fluorouracil to inactive molecules, could be useful in predicting the response of patients with metastatic gastric cancer to chemotherapy using 5′-DFUR. Endoscopic biopsy specimens for the measurement of TP and DPD were obtained from the primary lesions before the start of combination chemotherapy, in which 5′-DFUR, cisplatin and mitomycin C were administered. TP and DPD were measured by enzyme-linked immunosorbent assays after the objective responses to chemotherapy had been confirmed. Twenty five patients were enrolled in this study and data for 22 patients in whom responses were confirmed were analysed. The median levels (ranges) of TP and DPD were 80 (4.9–360) and 44 (15–82) U/mg protein, respectively. The median value (range) of TP to DPD ratios was 1.9 (0.25–5.1). Eight patients with a complete or partial response to chemotherapy had significantly higher TP to DPD ratios than did the remaining patients with stable or progressive disease ($P = 0.014$). When a cut-off level of TP to DPD ratio was defined as the median value, the high-ratio group ($n = 11$) showed a significantly higher response rate (64% vs. 9.1%, $P = 0.024$) than the low-ratio group ($n = 11$). Overall survival of the high-ratio group was significantly longer than that of the low-ratio group (the median survival time; 300 days vs. 183 days, $P = 0.047$). The efficacy of 5′-DFUR could be optimised by preselecting patients with high TP/DPD ratios in their tumour tissues, and this would be applicable to the treatment with capecitabine.

Keywords: Thymidine phosphorylase; Dihydropyrimidine dehydrogenase; Gastric cancer; 5′-Deoxy-5-fluorouridine; Capecitabine; Mitomycin C; Cisplatin; ELISA

1. Introduction

Many methods for predicting the susceptibility of a cancer to various chemotherapy regimens have been investigated. One of the best known is chemosensitivity testing by culturing of tumour cells with the chemotherapeutic agents [1,2]. Another useful approach is the analysis of enzymes involved in the activation or inactivation of chemotherapeutic agents. However, the clinical relevance of such tests has not been established.

Thymidine phosphorylase (TP) is an enzyme involved in pyrimidine nucleoside metabolism. It has been

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recently reported that TP is identical to platelet-derived endothelial cell growth factor and has been implicated in angiogenesis [3–5]. It has also been reported that high expression of TP in tumours was indicative of a poor prognosis [6]. TP is expressed in a wide variety of solid tumours (carcinomas of the breast, stomach, colon, pancreas and lung), and its content is higher in tumour tissues than in adjacent normal tissues [7,8]. Capecitabine and 5′-deoxy-5-fluorouridine (5′-DFUR), which is an intermetabolite of capecitabine, are oral prodrugs of 5-fluorouracil (5-FU), and TP is an essential enzyme that converts them to 5-FU [9,10]. High amounts of TP in tumours are suggested to enhance the efficacy of 5′-DFUR [11]. 5′-DFUR is currently being used for the treatment of gastric cancer in Japan [12,13]. Conversely, 5-FU is catabolised to biologically inactive molecules such as dihydrofluorouracil by dihydropyrimidine dehydrogenase (DPD) and DPD reduces the efficacy of 5-FU against tumours [14–16].

Recently, it was revealed that the efficacy of 5′-DFUR was correlated with the ratio of TP to DPD (TP/DPD) activity in human cancer xenograft models [17]. Furthermore, a clinical study using 5′-DFUR in adjuvant chemotherapy showed that patients with a high TP/DPD ratio in gastric cancer tissues had longer disease-free survival than did the patients with a low TP/DPD ratio [18]. Thus, the efficacy of 5′-DFUR is strongly influenced by the contents of TP and DPD in the tumour tissues. Recently, convenient enzyme-linked immunosorbent assays (ELISA) for measuring TP and DPD in human cancer tissues were developed [7,8]. The TP and DPD contents determined by each ELISA showed good correlation in clinical samples of tumour tissues with those determined by enzyme-activity assays [7,8]. This enabled us to measure those enzymes quantitatively in small portions of biopsy specimens, some comprising as little as 10 mg of tissue.

On the basis of these data, we conducted a prospective multicentre trial to investigate the relation between TP and DPD in tumour tissues measured by ELISA and the efficacy of 5′-DFUR.

2. Materials and methods

2.1. Patient population and eligibility

Patients were eligible for this study if they met the following inclusion criteria: (a) Patients having histologically proven gastric adenocarcinoma; (b) patients with advanced or recurrent gastric cancer with measurable metastatic lesions; (c) biopsy specimens taken from the primary lesion for the TP and DPD assays; (d) patients not less than 20 years old; (e) performance status of 0–2 (Eastern Cooperative Oncology Group); (f) no prior 5′-DFUR administration; (g) no severe major organ dysfunction; (h) no severe complications; (i) expected to survive more than 8 weeks and (j) written informed consent obtained.

The institutional review board of each participating institution approved this study.

2.2. Treatment methods

The treatment regimen was as follows: 5′-DFUR, 1200 mg/m^2 per day, was given orally from days 1 to 5; cisplatin, 10 mg/m^2 per day, was given by 30-min intravenous drip infusion on days 1 and 4; mitomycin C (MMC), 5 mg/m^2 per day, was given by bolus intravenous injection on day 8. This treatment was repeated every 2 weeks and was continued until progression or until occurrence of unacceptable adverse reactions.

2.3. Response criteria and treatment evaluation

The response to treatment was evaluated according to revised World Health Organisation criteria (Response Evaluation Criteria in Solid Tumours) every 4–6 weeks [19]. The response of each patient to the treatment was assessed by a group of extramural reviewers. All adverse reactions were graded according to the National Cancer Institute Common Toxicity Criteria version 2.

2.4. Preparation of biopsy specimens

Fresh endoscopic biopsy specimens (at least five biopsy samples from each patients) taken for the measurement of TP and DPD were sampled from primary lesions before the start of chemotherapy, after informed consent had been obtained. Samples from each patient were immediately frozen and stored at −80 °C. After the response to chemotherapy had been confirmed, each specimen was homogenised in a 10-fold volume of 10 mM Tris–HCl buffer (pH 7.4) containing 15 mM NaCl, 1.5 mM $MgCl_2$ and 50 μM potassium phosphate, then centrifuged at 10,000*g* for 15 min. The supernatant was stored at −80 °C. The protein concentration in the supernatant extracted from the tumour tissue was determined using a DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA).

2.5. TP ELISA

TP in tumour tissues was measured by ELISA [7], and the enzyme contents were expressed as U/mg protein, where 1 U was equivalent to the amount of TP generating 1 μg of 5-FU in 1 h. The interassay precision of TP ELISA had a coefficient of variation (CV) of 8.6%.

2.6. DPD ELISA

DPD in tumour tissues was measured by a sandwich ELISA using two monoclonal antibodies specific to human DPD [8]. Enzyme contents were expressed as U/mg protein, where 1 U was equivalent to the amount of DPD catabolising 1 pmol of 5 FU/min. The interassay precision of DPD ELISA had a CV of 2.5%.

2.7. Statistical analysis

Differences among TP and DPD contents and TP/DPD ratios were analysed using the Mann–Whitney U test. Differences in response rates were analysed using Fisher's exact test. Overall survival rate was determined using the Kaplan–Meier method, and the log-rank test was used to calculate the difference in survival between the groups. P-values $\leqslant 0.05$ were regarded as statistically significant. All analyses were performed using SPSS software (version 11.5J; SPSS Inc., Tokyo).

3. Results

3.1. Case analysis and background factors

A total of 25 eligible patients was enrolled in the study between April 1999 and March 2002. Three patients could not have their responses evaluated because 5′-DFUR could not be administered sufficiently, due to gastrointestinal stenosis, and their treatments were terminated or changed too early. Responses to the chemotherapy were confirmed for 22 patients, and TP and DPD in the biopsy specimens from these patients were determined by ELISA. The backgrounds of these patients are shown in Table 1.

Table 1
Patient characteristics

Characteristics	Number of patients ($n = 22$)
Age (years)	
Median	66
Range	32–78
Sex	
Male	13
Female	9
Performance status (ECOG)	
0	10
1	10
2	2
Prior treatment	
Chemotherapy	3
Target lesion	
Lymph node	18
Liver	7
Lung	1
Histopathological type	
Differentiated	12
Undifferentiated	10

ECOG, Eastern Cooperative Oncology Group.

3.2. Response to chemotherapy and toxicity

The responses to treatment were: complete response (CR) one, partial response (PR) seven, stable disease (SD) six and progressive disease (PD) eight. The overall response rate was 36% (8/22). Grade 3 or 4 toxicity was caused by anorexia (4%), neutropenia (28%), anaemia (8%) and thrombocytopenia (12%). There were no treatment-related deaths.

3.3. Relationship between TP and DPD and responses to chemotherapy

The values of TP, DPD and TP/DPD ratios for each patient are plotted in Fig. 1(a), (b) and (c), respectively. The median TP for all 22 patients was 80 U/mg protein (range 4.9–360 U/mg protein) and that of DPD was 44 U/mg protein (range 15–82 U/mg protein). The median TP/DPD ratio was 1.9 (range 0.25–5.1). Neither TP nor DPD contents were significantly different between the responder (CR + PR) and the non-responder (SD + PD) groups ($P = 0.25$ and $P = 0.23$, respectively) (Fig. 1(a) and (b)). There was a considerable overlap for the distribution of TP/DPD ratios between responders and non-responders, but the ratios were significantly higher in the responder group than in the non-responder group ($P = 0.014$) (Fig. 1(c)). When a cut-off level for TP and DPD contents was assigned as the median value, there were no significant differences in response rates between the high- and low-level groups (response rate for TP, 55% vs. 18%, $P = 0.18$; response rate for DPD, 27% vs. 46%, $P = 0.66$). However, when the median value of the TP/DPD ratios was designated as a cut-off level, the high TP/DPD ratio group had a significantly higher response rate than did the low ratio group (64% vs. 9.1%, $P = 0.024$) (Table 2).

There was no significant correlation between the TP/DPD ratios and the severity of toxicities.

3.4. Survival

Only one patient was alive for 630 days up to the final follow-up time and the remaining patients were all dead due to tumour progression. The overall median survival time (MST) was 240 days. When each cut-off level for TP and DPD was assigned as the median value, there were no significant differences in survival between the high- and low-level groups (MST for TP, 207 days vs. 284 days, $P = 0.91$; MST for DPD, 240 days vs. 207 days, $P = 0.62$). When the median value

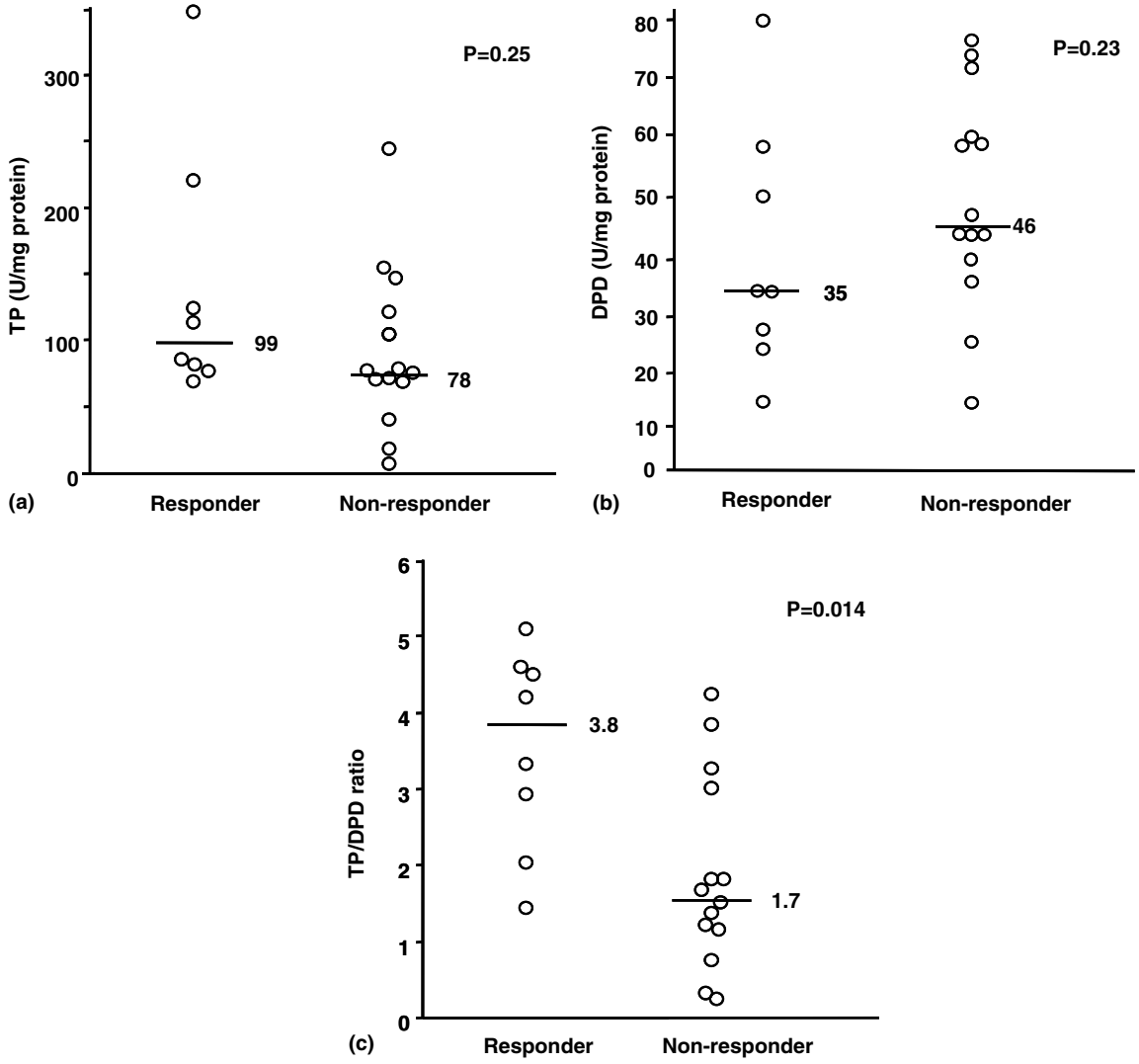

Fig. 1. Comparison of thymidine phosphorylase (TP) (a) and dihydropyrimidine dehydrogenase (DPD) (b) contents (U/mg protein) and TP/DPD (c) ratios in tumour tissues for the responder and non-responder groups. No significant difference between the groups was found for TP ($P = 0.25$, Mann–Whitney U test) and DPD ($P = 0.23$, Mann–Whitney U test) contents. The TP/DPD ratio was significantly higher in the responder group ($P = 0.014$, Mann–Whitney U test). Short horizontal line indicates the median value of each parameter in responder and non-responder groups.

of the TP/DPD ratios was designated as a cut-off level, the MST was 300 days for the high-ratio group and 183 days for the low-ratio group (Fig. 2). There was a marginal, but significant, difference between the two groups ($P = 0.047$).

4. Discussion

The biologically active drug 5-FU is formed selectively from 5′-DFUR by enzymatic conversion in TP-rich tumour tissues [10]. However, 5-FU is subsequently

Table 2
Response and TP/DPD ratios

TP/DPD	Response				Response rates (%)	95% CI
	CR	PR	SD	PD		
High ratio ($\geqslant$ 1.9)	1	6	3	1	64*	31–89
Low ratio (<1.9)	0	1	3	7	9.1*	0.2–41

CI, confidence interval.
CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.
* $P = 0.024$ (Fisher's test).

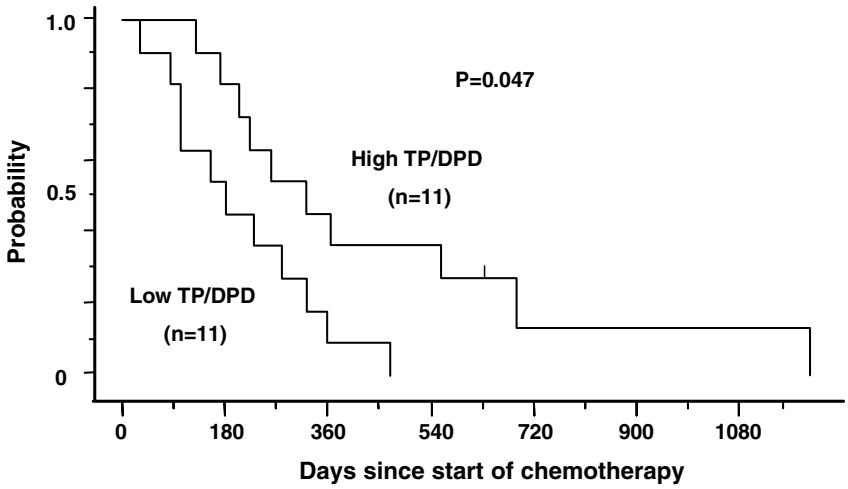

Fig. 2. Survival plots for patients with high and low thymidine phosphorylase/dihydropyrimidine dehydrogenase (TP/DPD) ratios in tumour tissues. Survival for the patients with high TP/DPD ratios was significantly longer than that for the patients with low ratios ($P = 0.047$, log-rank test).

catabolised to inactive molecules by DPD [14,15]. We showed a significant correlation between the ratios of these enzymes in tumour tissues and the clinical response to 5′-DFUR with improved survival in patients with metastatic gastric cancer, although the sample size was small.

In this study, although it was difficult to predict the response to 5′-DFUR based on either TP or DPD contents alone, the TP/DPD ratio in tumour tissues was a good predictor of tumour response to chemotherapy for metastatic gastric cancer. These data are consistent with the recent reports that the TP/DPD ratio in tumours was a better predictor of susceptibility to 5′-DFUR than either TP content or DPD content alone in human cancer xenograft models [17] and in a clinical study of adjuvant therapy for advanced gastric cancer [18].

There are many methods for the measurement of TP and DPD, including immunohistochemistry, a transcription-polymerase chain reaction method and an enzyme-activity assay [7,11,20]. The immunohistochemical method is the most widely used because of its convenience, but it is difficult to measure the amounts of TP and DPD quantitatively. In stead, we used ELISA to measure TP and DPD in this study. This method is also convenient and allows the quantification of enzyme activity in small pieces of tissue such as endoscopic biopsy specimens. These quantitative data allow the investigators to judge the results objectively. However, this method contains some weakness. All of the biopsy specimens taken may not be always tumour tissues, and intratumoural heterogeneity in TP content has been reported [21]. To avoid these biases in sampling, more than five biopsy specimens were obtained carefully from the same site of the primary lesion at which the diagnosis of adenocarcinoma had been confirmed by previous endoscopy. Another concern of this study is that TP and DPD contents of primary lesions were used as the predictor for the response, although the lesions that were used as indicator lesions for the response assessment were predominantly lymph nodes or liver metastases. To the best of our knowledge, there are no reports that TP/DPD ratios in primary tumour tissues correspond to those in metastatic lesions. Our data demonstrate that the measurement of TP and DPD enzyme contents in the primary tumour tissue could help in predicting the response to 5′ DFUR in metastatic disease sites.

Phase II studies using 5′-DFUR in Japan have shown a response rate of 14.3% (20/140) for patients with inoperable gastric cancer [12]. One promising approach for optimising therapy would be to combine 5′-DFUR with other agents, such as upregulators of TP and other biochemical modulators. Several anticancer drugs, including MMC, upregulate the expression of TP, and MMC in combination with 5′-DFUR shows synergistic activity in several human cancer xenograft models [22]. Cisplatin combination therapy affects biochemical modulation with 5-FU; cisplatin increased the availability of the reduced folate for tight binding of the 5-fluorodeoxyuridylate-generated form 5-FU to thymidylate synthase (TS) and enhanced the efficacy of 5-FU [23]. Therefore, in our present study, these drugs were combined to enhance the antitumour activity of 5′-DFUR. In fact, our study yielded a 36% response rate. This result was similar to those in several previous reports of 5′-DFUR combination therapies [13,24].

MMC upregulates the expression of TP in tumour tissues. Cisplatin reportedly has no effect on the expression of TP [22]. It is unclear how MMC or cisplatin influence the expression of DPD. Although we cannot exclude the possible influence of adding MMC and cisplatin on our finding that the TP/DPD ratio was predictive of response to 5′-DFUR, previous studies on the

expression of TP/DPD and the response to 5′-DFUR in experimental human xenograft models and in the clinical adjuvant chemotherapy setting strongly supports our conclusion [17,18]. Recently, capecitabine has been developed as a new fluoropyrimidine carbamate drug. It is a prodrug of the 5′-DFUR used in this study. The total amount of 5-FU generated from capecitabine within the tumours was 2.8- to 4.3-fold higher than that from 5′-DFUR [25]. High response rates have been reported recently for the treatment of gastric cancer with capecitabine [26,27]. Taking together with our findings, the response to capecitabine would be predicted more precisely by the use of TP/DPD ratio in the gastric cancer tissues. We are planning a clinical study to investigate the relative efficacies of capecitabine and the enzyme contents of TP and DPD, adding TS, which is the target of 5-FU and one of the predictors of its efficacy.

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