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Clinical report

Dihydropyrimidine dehydrogenase-related enzymes predict efficacy and adverse reactions of UFT+cisplatin neoadjuvant chemotherapy for gastric cancer

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Dihydropyrimidine dehydrogenase (DPD) and dihydropyrimidinase (DHP) are metabolic enzymes of fluoropyrimidine. UFT containing uracil (U) and Tegafur is the first reported DPD-inhibitory fluoropyrimidine. To clarify the significance of the enzyme activities, we examined the relationships between the effects and adverse reactions, and DPD and DHP activities in gastric cancer treated with UFT+cisplatin neoadjuvant chemotherapy. Twenty-five gastric cancer patients were administered UFT at 370 mg/m^2/day for 21 days and cisplatin at 15 mg/m^2/day for 2 days. Dihydrouracil (DU) and U levels in the urine and DPD activities in the resected tumors were measured. Chemotherapeutic effects were classified histologically into non-responder and responder groups. The responder group accounted for 48% of the patients. All six patients with high DPD activities ($\geq$0.08 nmol/min/ww) belonged to the non-responder group and 11 of 19 patients with low DPD activities (<0.08 nmol/min/ww) belonged to the responder group; the difference was significant (p=0.0435). Adverse reactions to UFT occurred in four patients, all of whom were among the six patients with abnormal DU/U values. The incidence of UFT adverse reactions was estimated at 67%. In conclusion, the measured levels of DPD-related enzyme activities appear to be significant for predicting the effects and adverse reactions to chemotherapy. [© 2002 Lippincott Williams & Wilkins.]

Key words: 5-Fluorouracil, dihydropyrimidinase, dihydropyrimidine dehydrogenase, gastric cancer, UFT.

Introduction

Fluorinated pyrimidine-type anticancer drugs are converted into 5-fluorouracil (5-FU) in the human body, and show cytotoxic effects through functional disturbances of RNA and inhibition of DNA synthesis in cancer cells. In the biosynthesis of DNA, thymidylate synthase (TS) catalyzes the methylation between deoxyuridine monophosphate (dUMP) and deoxythymidine monophosphate (dTMP). This is the only *de novo* synthesis pathway of deoxyuridine phosphoric acid (dTTP), the substrate for DNA synthesis.[1]

However, most of the 5-FU produced from administered fluorinated pyrimidine-type anticancer agents is converted to inactive fluoro-β-alanine by metabolic enzymes such as dihydropyrimidine dehydrogenase (DPD) and dihydropyrimidinase (DHP), and is excreted in the urine without showing any cytotoxic effects.[2,3] TS, DPD and DHP serve as indices of the effects and adverse reactions to anticancer agents. UFT, which is metabolically regulated by DPD and DHP, is an oral drug formulation containing uracil (U) and Tegafur in a molar ratio of 4:1.[4,5] Biochemical modulation of U enhances the 5-FU concentration more specifically within tumor tissues than in normal tissues.[1,6] Cisplatin also functions as a biochemical modulator for 5-FU.[7] Neoadjuvant chemotherapy has been advocated for the treatment of advanced gastric cancer to improve curative resection rates by down-staging and leading to fewer distant metastases.[8] In the current study, we examined the relationships between the chemotherapeutic effects and adverse reactions to co-administration of UFT and cisplatin, and DPD and DHP activities in patients undergoing neoadjuvant chemotherapy for gastric cancer.

Materials and methods

Twenty-five gastric cancer patients preoperatively diagnosed with tumor invasion into or beyond the

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proper muscle layer according to endoscopic ultrasonography were treated with UFT-based neoadjuvant chemotherapy between August 1997 and August 1999. The protocol of neoadjuvant chemotherapy was that UFT was administered orally at 370 mg/m^2/day for 21 days and cisplatin was administered i.v. at 15 mg/m^2/day for 2 days (day 1 and day 2). Informed consent for neoadjuvant chemotherapy was obtained from all patients and 25 gastric cancer patients were registered. This study was carried out with the approval of the Ethics Committee.

Measurement of DPD activity in resected specimens of tumor tissue and normal tissue

At least 500 mg of fresh tissue specimens of tumor and normal mucosa (from a distant site) obtained during gastrectomy were cryopreserved. After the tissues were weighed, buffer was added and they were homogenized. The supernatant obtained by centrifugation was filtered and the filtrate was used as the enzyme solution [pretreatment].

Substrates 5-FU (2 μg/ml) and NADPH (2 mM) were added to the enzyme solution, and the mixture was incubated at 37°C and shielded from light. After 0, 10, 20 and 30 min, a reaction-stopping solution and a 5-FU internal standard were added [enzyme reaction].

After sialylation with *N*,*O*-bistrifluoroacetamide, the concentration of 5-FU in the enzyme solution was measured by gas chromatography–mass spectrometry (GC-MS).

DPD activity=5-FU concentration/reaction time/amount of sample tissue (nmol/min/ww).

Estimation of DPD and DHP activity by measurement of U and dihydrouracil (DU) in urine

Most 5-FU is converted to inactive fluoro-β-alanine by metabolic enzymes such as DPD and DHP, and is excreted in the urine. U in food is metabolized into DU by DPD and DU is converted to ureidoproprionic acid (UPA) by DHP. UPA is converted to β-alanine by β-alanine synthetase, with the release of ammonia and CO_2. By measuring the amounts of U and DU in the urine, it is possible to predict the enzyme activity of DPD and DHP.[9] Prediction of the enzyme activity of DPD and DHP is considered to be reflected in the metabolism of 5-FU, which is metabolized via the same pathway. Since U is present in UFT itself and U in UFT functions as a competitive inhibitor of DPD, urine samples were collected in the morning before starting the neoadjuvant chemotherapy. Collected urine samples were cryopreserved and measured at Otsuka Assay Laboratory (Tokushima, Japan). The amounts of DU and U in the urine were measured by high-performance liquid chromatography (HPLC).[9] DPD and DHP were estimated from DU and U levels (standard value DU: 10.7–34.5 μmol/g Cr; U: 25.1−99.8 μmol/g Cr) or the DU/U ratio (standard value: 0.21–0.74). When the DU/U ratio is small, the DPD activity is assumed to be low, and when the ratio is high, the DHP activity is low.

Evaluation of the histological effects

The effects were assessed based on the Regulations for the General Rules for the Gastric Cancer Study.[10] Grade 1a in which the histological effects were evaluated at a third or less tumor cells diminishing, was subdivided into grade 1a(−) for weak cell degeneration and grade 1a(+) for potent cell degeneration. Grade 1a(−) or lower is considered a non-responder and grade 1a(+) or higher is considered a responder.

Statistical analysis

The statistical analysis was performed using the χ^2-test, paired *t*-test and Mann–Whitney *U*-test. $p<0.05$ was considered significant.

Results

DPD activity in tumor tissues and normal tissues in resected stomach specimens

DPD activity in tissue specimens after gastrectomy was significantly higher in normal tissues than in tumor tissue (paired *t*-test: $p=0.045$) (Figure 1). Correlation was observed at a Pearson correlation coefficient of 0.6607 ($p=0.0001$) (Figure 2).

DPD activity and histological effects and adverse reactions

As to the histological effects, only five patients (20% of all patients) belonged to grade 1b or higher. Fourteen patients belonged to grade 1a(−) or lower. All six patients with high DPD activities (0.08 nmol/min/ww or higher) belonged to grade 1a(−) or lower

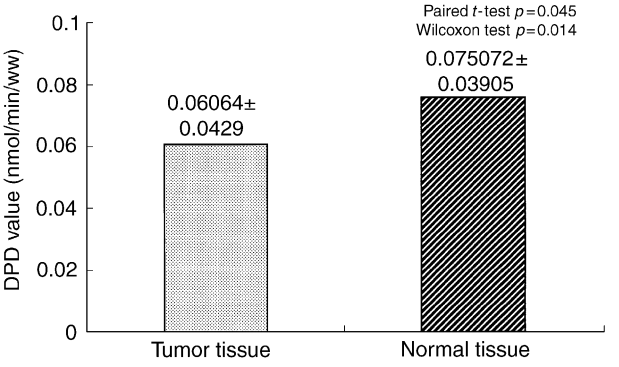

Figure 1. DPD activity levels of the tumor and normal tissue.

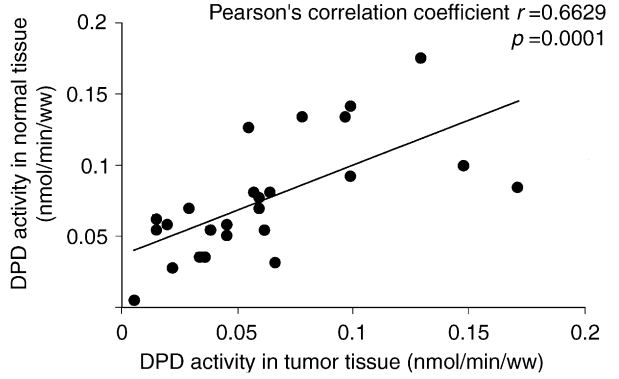

Figure 2. Correlation of DPD activity levels between the tumor and normal tissue.

and 11 of 19 patients with low DPD activities (less than 0.08 nmol/min/ww) belonged to grade 1a(+) or higher. The difference was significant at $p=0.0435$. This finding indicates that DPD activity influences the histological effects (Figure 3 and Table 1). However, there was no correlation between DPD activity in normal tissue and histological effects (Table 2).

DPD activities in normal tissues were 0.0577 ± 0.0007 nmol/min/ww with adverse reaction and 0.0784 ± 0.0406 nmol/min/ww without adverse reaction. DPD activities in tumor tissues were 0.0827 ± 0.0037 nmol/min/ww with adverse reaction and 0.0564 ± 0.0086 nmol/min/ww without adverse reaction. Thus there was no relationship between DPD activities in normal tissues or tumors, and adverse reactions.

Estimated expression of chemotherapeutic effects and adverse reactions from the ratio of U to DU excretion in urine

The excretion of U to DU in urine was measured in 24 patients. Nine of these patients showed adverse

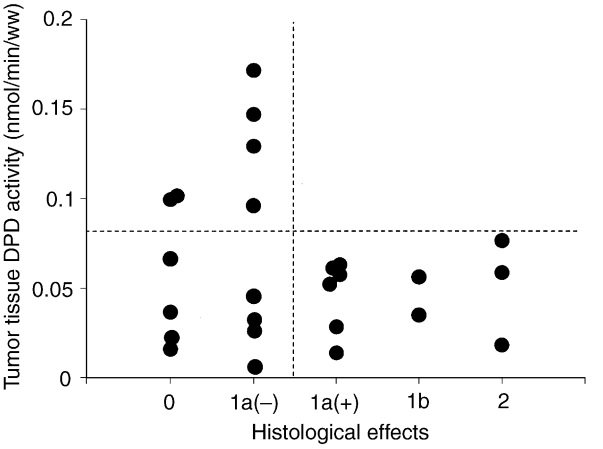

Figure 3. Tumor tissue DPD activity and histological effect in each patient.

Table 1. DPD activity of the tumor tissue and histological effects

DPD activity (nmol/min/ww)	Histological effects	
	Grade 1a(−) or lower	Grade 1a(+) or higher
⩾0.08	6	0
<0.08	8	11

$p=0.0435$ (χ^2-test).

Table 2. DPD activity of the normal tissue and histological effects

DPD activity (nmol/min/ww)	Histological effects	
	Grade 1a(−) or lower	Grade 1a(+) or higher
⩾0.08	6	3
<0.08	6	8

$p=0.6974$ (χ^2-test).

reactions. Nausea, vomiting and anorexia, which were considered to be adverse reactions to cisplatin and which had disappeared by the second week, occurred in five patients. Adverse reactions caused by UFT that appeared after the second week or that persisted beyond the second week were observed in four patients. All of these four patients were among the six patients with abnormal DU/U values, and high U and DU values. They included one case of stomatitis (grade 2) and two cases of leukopenia (both grade 1; one patient had both stomatitis and leukopenia). Two patients had diarrhea of grade 1, but there were no serious adverse reactions. It was

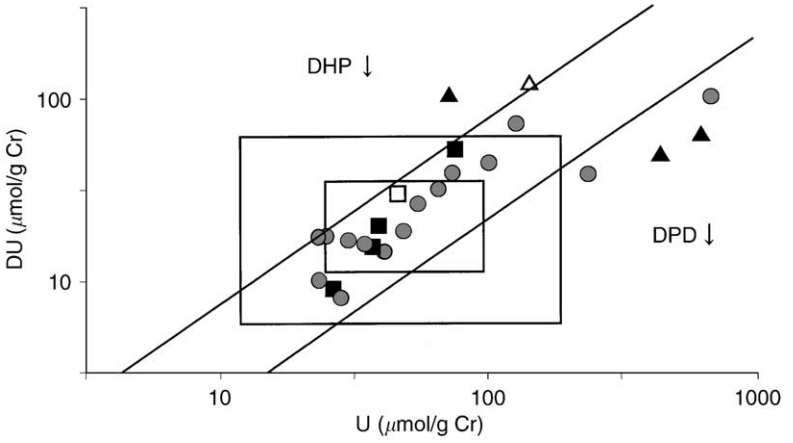

Figure 4. U/DU excretion in urine and adverse reactions. Shaded circles: no adverse reactions. Adverse reactions to UFT: filled triangles, grade 1; open triangles, grade 2. Adverse reactions to cisplatin: filled squares, grade 1; open squares, grade 2.

impossible to predict the histological effects from the DU/U ratio (Figure 4).

Discussion

In recent years, the biochemical mechanism of action of oral fluorinated pyrimidine anticancer agents in cancer cells has been clarified in detail and there have been many studies on the usefulness of combination chemotherapy for biochemical modulation using various modulators to reinforce the clinical antitumor effects.

UFT is the first reported DPD-inhibitory fluoropyrimidine (DIF).[4,5] A phase II study of UFT was conducted using a total daily dose of 600 mg/day for gastric cancer in Japan and the response rate was 27.7%.[11] In the west, recently developed oral anticancer agents such as S-1[5] and capecitabine[12] have been proven effective. Attention is now being focused on S-1 and UFT as DIFs. Oral anticancer agents, which make outpatient treatment possible, should occupy an important position in the future use of anticancer agents.

Currently, cisplatin is promising as a biochemical modulator for 5-FU. Cisplatin used concomitantly with 5-FU not only inhibits DNA synthesis, but also inhibits the uptake of methionine by the cells and increases the amount of reduced-type folic acid in cancer cells.[13]

Sato *et al*. reported a combination therapy with UFT and cisplatin for advanced gastric cancer.[14] UFT was administered orally at 400 mg/m^2/day for 28 days and cisplatin was given i.v. at 30 mg/m^2/day for 3 days every 4 weeks. This treatment cycle was repeated every 4 weeks. The response rate of this study was 42.9% and high-grade toxicity of the intestinal tract was seen in more than 30% of the patients.

Concerning the clinical effects of preoperative administration of UFT, Sato *et al*. reported the results of oral administration of 300–600 mg/day of UFT to 32 patients with gastric cancer for 2–3 weeks on consecutive days until the day before the operation.[15] In their study, the response rate of macroscopic criteria was 38.5% and the histological response rate of grade 2 or higher was 18.8%.

In consideration of these findings, we administered the neoadjuvant chemotherapy with UFT+cisplatin for advanced gastric cancer. We tried to clarify the relationships among the effects of the chemotherapy and the 5-FU metabolism-related enzymes such as DPD and DHP in these patients.

Our findings on the histological effects of neoadjuvant chemotherapy showed that in spite of cisplatin co-administration, the grade 1b or higher response rate was only 20%, which was lower than Sato's finding (37.5%).[14] Although the histological effect was diagnosed as grade 1a because of the disappearance of cancer cells to less than one-third of their original number, the tumor cell degeneration was divided into marked and unremarkable. Furthermore, the number of grade 1b cases or higher was low, as we classified the patients into grade 1a(+) with potent cell degeneration and grade 1a(–) with little cell degeneration. The patients of grade 1a were divided into eight cases of grade 1a(–) and six cases of grade 1a(+). To achieve histological effects of grade 1a(+) or higher, DPD activity in tumor tissues of 0.08 nmol/min/ww or less was necessary. This

finding shows that, in spite of UFT chemotherapy, the importance of the low activity of DPD in tumor tissues is in fact predictive of efficacy. Biochemical modulation of U enhances the 5-FU concentration more specifically within tumor tissues functioning as a competitive inhibitor of DPD. DPD activities were revealed to be important for prediction of chemotherapeutic effects by fluorinated pyrimidine-type anticancer drugs containing DIF. There are few reports in the literature on the DPD activity cut-off value,[16] but in this study the responder and non-responder groups could be clearly distinguished at a cut-off value of 0.08 nmol/min/ww.

We also investigated the correlation between DPD activity in tumor and normal tissues in the stomach. The activity in tumor tissues tended to be lower than that in normal tissues, but there was a correlation between them in that for patients with high values in tumor tissues, the values tended to be high in normal tissues. However, the histological effects of 5-FU could be evaluated from the DPD activity in tumor tissues, but not in normal tissues. Reports of DPD activity in tumors is definitely higher than in normal tissues in breast cancer patients,[17] but for colorectal cancer[18] and stomach cancer[19] there have been conflicting reports of no significant difference and of significantly higher activity in tumor tissues. The 5-FU concentration in normal tissues was less than half of that in tumor tissues.[6] Therefore, DPD activity in tumor tissues is useful in the prediction of efficacy. Our data showed the prediction of adverse drug reaction by normal tissues and tumor tissues was difficult. This indicates that it is difficult to predict systemic adverse reactions from local DPD activity.

DPD and DHP show the highest metabolic activity in the liver, and systemic DPD and DHP activity can be predicted from the levels of U and DU excretion in urine.[9] Since the U in UFT is intended to function as a competitive inhibitor of DPD and the activity of DPD varies several-fold throughout the day for individual patients, urine samples were collected in the morning before starting the chemotherapy. In this study, we found that measured DU/U ratio in urine did not correlate to DPD activity in the tumor specimens. There was also no relationship with the antitumor effects of UFT. However, in the prediction of adverse reactions to UFT, six patients had high values of U and DU in urine and an abnormal DU/U ratio, and adverse reactions to UFT were predicted in four of these patients (prediction rate: 67%). Patients with normal DU/U ratios did not have any adverse reactions to UFT.

Sumi *et al.* have previously reported the measurement of U and DU. According to their findings, the measurement of U and DU is considered to be easy for the detection of DPD deficiency or DHP deficiency and important in predicting adverse reactions.[20,21] The levels of DPD-related enzymes were also shown to be predictive of the effects and adverse reactions to 5-FU type chemotherapy to a certain extent in our study.

However, in this protocol for gastric cancer, the histological effects were not sufficient and it is necessary to further increase the antitumor effects of oral 5-FU type anticancer drugs. These results suggest that UFT may have insufficient antitumor effect under the influence of high DPD activity in tumor tissue. DIF drugs such as S-1, which is stronger than UFT because it has 200-fold DPD-inhibitory activity, are considered to be more effective because they maintain concentrations of 5-FU in tumor tissue.

Conclusion

Twenty-five advanced gastric cancer patients were treated with UFT+cisplatin neoadjuvant chemotherapy. UFT was administered orally at 370 mg/m^2/day for 21 days and cisplatin was administered i.v. at 15 mg/m^2/day for 2 days. DPD activity of the tumor was thought to be one of the predictive factors for the response to UFT based chemotherapy.

Our study showed that the measured DU/U ratio in urine did not correlate to DPD activity in the tumor specimens and there was also no relationship with the antitumor effects of UFT. However, adverse reactions to UFT were thought to be predictable by measured values of U and DU in urine and an abnormal DU/U ratio (prediction rate: 67%). However, in this protocol for gastric cancer, the histological effects were not sufficient and it is necessary to further increase the antitumor effects of oral 5-FU-type anticancer drugs.

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