ORIGINAL

Thymidine phosphorylase and dihydropyrimidine dehydrogenase are predictive factors of therapeutic efficacy of capecitabine monotherapy for breast cancer-preliminary results

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Abstract : Capecitabine monotherapy was administered for 25 patients with advanced or recurrent breast cancer, and the clinical therapeutic efficacy and its relationship to expression of 5-fluorouracil-related enzymes (i.e., thymidine phosphorylase (TP), thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD)) were investigated. The expressions of TP, TS and DPD were determined by immunohistochemical staining techniques and rated using a scoring system of 1~4. The expression score for TP/DPD showed a statistically significant correlation with the clinical response, whereas the expression score for TP/TS also showed a correlation but it was not statistically significant. The number of patients was small, but the results revealed the potential of application of the TP/DPD expression score as a factor for predicting the efficacy of the drug in individual patients. J. Med. Invest. 55 : 54-60, February, 2008

Keywords **:** *breast cancer, capecitabine, dihydropyrimidine dehydrogenase, predictive factor, thymidine phosphorylase*

INTRODUCTION

The results of clinical research on the treatment of breast cancer have elucidated factors that predict the efficacy of a therapy, thus enabling individualization of the therapeutic approach. In particular, individualized regimens have been established for patients who are positive for estrogen receptor (ER) or progesterone receptor (PgR) and patients who show overexpression of the c-erbB2 gene or its products (also known as HER2/neu) (1-3). On the other hand, there is no consensus regarding the chemotherapy regimen for breast cancer, although there have been reports of efficacy with regimens including an anthracycline in patients who overexpress HER2 (4). In addition, thymidine phosphorylase (TP), thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD) have also been studied as predictive factors for treatment with 5-fluorouracil (5-FU) and its derivatives (5, 6). There

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are various methods for assay of those enzymes, including quantitative determination of their activities, the reverse transcript-polymerase chain reaction (RT-PCR) to determine the mRNA level and immunohistochemical staining methods (7). Quantitative determination of the enzyme activity and RT-PCR are the best methods for obtaining accurate information regarding these enzyme activities, but they require fresh samples and are technically complicated. In contrast, the immunohistochemical staining method is technically simple, and although ordinary samples are sufficient for assay the results are only qualitative.

In this study, we administered capecitabine, a 5-FU derivative, monotherapy to Japanese patients with advanced or recurrent breast cancer, performed immunohistochemical staining on surgical specimens and semi-quantitatively determined the expression levels of TP, TS and DPD by applying a scoring method to the staining results (8). Then the scores for the enzyme expression levels were analyzed for correlations with the clinical response to capecitabine.

PATIENTS AND METHODS

Patients

During the period from September 2003 through December 2006, 25 Japanese women with advanced or recurrent breast cancer were administered capecitabine monotherapy at Tokushima Breast Care Clinic. The clinical efficacy of the treatment in those patients was able to be evaluated. The patients consisted of three with advanced disease and 22 with recurrent disease. The capecitabine dosage was 2,400 mg/day for a body surface area of 1.31~1.46 m^2, 1,800 mg/day for a body surface area of less than 1.31 m^2 and 3,000 mg/day for an area of greater than 1.46 m^2. The clinical efficacy of the treatment was assessed in accordance with the General Rules for Clinical and Pathological Recording of Breast Cancer (15th Edition) (The Japanese Breast Cancer Society) (9). The following clinicopathological parameters were investigated for each of the patients : the age, disease free interval (DFI), content of prior therapy, status of ER and PgR, and expression of HER2. The investigations of ER, PgR and Her2 expressions and the assessment of the findings were carried out by the conventional immunohistochemical staining techniques.

We started this study after having got informed consent in the word of mouth from each patient or a family of a patient.

Immunohistochemical staining for TP, TS and DPD

Formalin-fixed, paraffin-embedded surgically resected specimens were subjected to immunohistochemical staining for each of TP, TS and DPD. The respective primary antibodies used were 1C6-203, TS106 and 2H9-1b, and the conventional avidin-biotin-peroxidase complex method was applied (Table 1). The staining results were rated using a four-stage scoring (1~4) system (8). That is, a score of 1 was assigned in the case of no staining or staining of less than 25% of the observed cells. A score of 2 was given to specimens for which there was slight staining of at least 25% of the cells, while a score of 3 was assigned to specimens showing weak staining of at least 25% of the cells and a score of 4 was given to specimens showing strong staining of at least 25% of the cells. TP staining is also observed in the nucleus of cells, and thus both the nucleus and cytoplasm were evaluated (Fig. 1). TS and DPD are detected only in the cytoplasm, and thus

Table 1. Sources, dilution, pretreatment of antibodies used

Antibody	Clone	Manufacturer	Dilution	Pretreatment
TP	1C6-203	Chugai	1 : 500	autoclave
TS	TS106	Quartett	1 : 25	autoclave
DPD	2H9-1b	Chugai	1 : 100	autoclave

TP : Thymidine phosphorylase, DPD : Dihydropyrimidine dehydrogenase, TS : Thymidine synthase

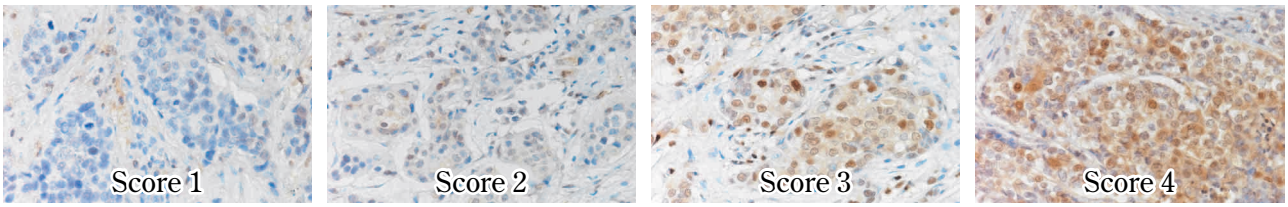

Fig. 1 Immunohistochemical staining for TP. TP staining is also observed in the nucleus of cells, and thus both the nucleus and cytoplasm were evaluated. That is, a score of 1 was assigned in the case of no staining or staining of less than 25% of the observed cells. A score of 2 was given to specimens for which there was slight staining of at least 25% of the cells, while a score of 3 was assigned to specimens showing weak staining of at least 25% of the cells and a score of 4 was given to specimens showing strong staining of at least 25% of the cells.

only the cytoplasm was evaluated for staining for these enzymes.

Correlations between clinical response and expressions of TP, TS and DPD

The clinical response of the patients and the scores for the expressions of TP, TS and DPD, singly and in combination, i.e., TP/DPD, TP/TS and TS/DPD, were analyzed for the presence of correlations.

Statistical analyses

All data are expressed as mean ± SD. Comparison of the clinical response to the capecitabine monotherapy and the patient background factors was performed using chi-square test. The clinical response of the patients and the scores for the enzyme expressions were analyzed for the presence of correlations using the t-test. A p value of <0.05 was considered statistically significant.

RESULTS

Clinical results

The age range of the patients was 35~79 years (mean : 57± 11.3 years), and the mean disease-free interval (DFI) was 11 months. The sites of recurrence in the 23 patients with recurrent disease were the lung in 5 patients, the liver in three patients, the bones in 10 patients, the lymph nodes in 14 patients (some patients had recurrence at multiple sites). The overall results of the capecitabine monotherapy showed a partial response (PR) in 5 patients, stable disease (SD) in 9 patients (including 5 patients with long SD of at least 6 months) and 11 patients with progressive disease (PD). The overall response rate (ORR) was 20% (5/25 patients), while the clinical benefit (PR plus long SD cases) rate was 40% (10/25 patients) (Table 2). Analysis of the therapeutic efficacy as a function of the site of recurrence showed one case of PR and 2 cases of SD in the 5 patients with recurrence in the lung. The 10 patients with recurrences in the bones showed one case of PR and 5 cases of SD, while 2 of the 3 patients with recurrence in the liver showed SD. The results in the 14 patients with recurrences in the lymph nodes showed 3 cases of PR and 7 cases of SD.

Table 2. Clinical results in 25 patients with recurrent or advanced breast cancer treated capecitabine monotherapy.

Clinical results	cases
Partial response (PR)	5
Stable disease (SD)	9
(long SD 5)	
Progressive disease (PD)	11
Clinical response	5/25 (20%)
Clinical benefit (PR + long SD)	10/25 (40%)

Correlations between therapeutic efficacy and patient background factors

Stratification of the therapeutic efficacy as a function of the background factors found no difference arising from the patients' age. However, the DFI was shorter in the patients showing PD compared with in the patients achieving PR or SD. Almost all of the patients had undergone chemoendocrine therapy prior to enrollment in the present study. Fourteen patients had been treated with taxan, and approximately half had shown a clinical benefit. In addition, 6 patients had been treated with a 5-FU derivative, and more than half of those patients also had shown a clinical benefit. Regardless of whether or not hormone therapy had been administered in the past, and the statuses for ER, PgR or Her2, there were no correlations with the drug response (Table 3).

Table 3. Correlations betweem therapeutic efficacy and patient background factors

Clinical response	PR	SD	long SD	PD
age	54.2± 9.3	58.4± 14.4	(53.2± 15.2)	57.1± 10
DFI	29± 19	20± 13	(19± 10)	13± 11
Previous treatment				
anthracycline	4	4	3	9
taxan	4	3	3	7
5-FU	2	2	0	2
hormone therapy	4	7	4	8
ER (+)	3	6	3	6
(–)	2	3	2	5
PgR (+)	3	4	2	5
(–)	2	5	3	6
Her2(0-1)	2	6	3	7
(2-3)	2	3	2	3

PR : partial response, SD : stable disease, PD : progressive disease, DFI : disease free inferval, ER : estrogen receptor, PgR : progesterone receptor

Correlations between therapeutic efficacy and TP, TS and DPD scores

The therapeutic results were stratified as a function of the TP, TS and DPD scores. The TP score showed a tendency to be higher in the PR and SD patients compared with the PD patients, but the differences were not statistically significant. The TS score did not show any effect on the therapeutic efficacy. The DPD score showed a tendency to be lower in the PR patients compared with the SD and PD patients, but again the differences were not statistically significant (Table 4).

Table 4. Correlations between therapeutic efficacy and TP, TS, DPD scores.

	PR	SD	long SD	PD
TP	3.0(2-4)	2.8(2-4)	2.0(2-4)	2.0(1-4)
TS	3.2(2-4)	3.4(3-4)	3.4(3-4)	3.3(2-4)
DPD	2.0(2-4)	2.6(1-3)	2.2(1-3)	2.4(1-3)

PR : partial response, SD : stable disease, PD : progressive disease, DFI : disease free inferval, ER : estrogen receptor, PgR : progesterone receptor

Correlations between therapeutic efficacy and combined scores for TS/DPD, TP/DPD and TP/TS

The combined score for TS/DPD showed no correlations with the drug response. Conversely, the combined score for TP/DPD was significantly higher in the order of PR, long SD and PD (Fig. 2). The combined score for TP/TS showed higher in the order of PR, long SD and PD, but there were no statistically significant differences (Fig. 3).

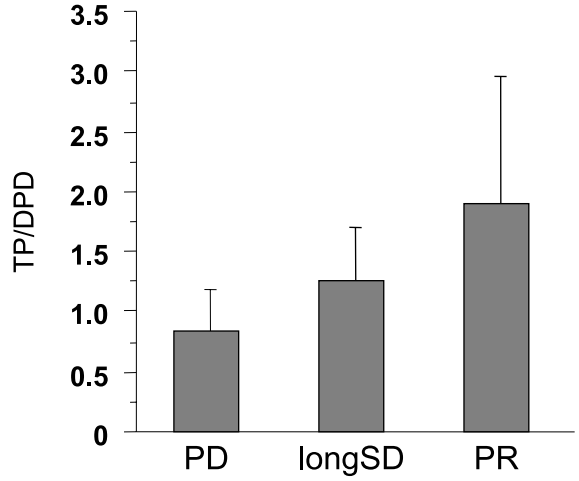

Fig. 2 Correlations between therapeutic efficacy and combined scores for TP/DPD. The combined score for TP/DPD was significantly higher in the order of PR, long SD and PD (PD vs PR : P < 0.01, PD vs long SD : P=0.05).

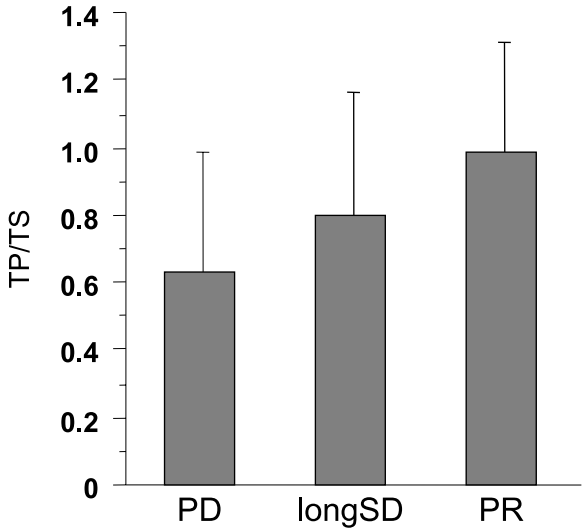

Fig. 3 Correlations between therapeutic efficacy and combined scores for TP/TS. The combined score for TP/TS showed higher in the order of PR, long SD and PD, but there were no statistically significant differences (PD vs PR : p=0.079).

DISCUSSION

Drugs for molecular-targeted therapy are being developed for the treatment of cancers, and in combination with proper selection of patients who are likely to respond the trend is toward improved response rates (1-4). In the treatment of breast cancer, high efficacy has been achieved with chemotherapy regimens including anthracyclines and with taxan, and these chemotherapy regimens are being employed both prior to surgery and as postoperative adjuvant chemotherapy (1). Other chemotherapeutic agents that have been effective in the treatment of breast cancer include vinorelbine and 5-FU derivatives, but they are generally used in patients with recurrent disease. Capecitabine is a 5-FU derivative that was developed as a prodrug of 5'-deoxy-5-fluorouridine (5'-DFUR) (10-12). It has been reported that TP, TS and DPD are involved in the activation/inactivation of 5-FU and its derivatives and are useful as factors in predicting the response of breast cancer patients to those drugs. TP is an enzyme that converts capecitabine and 5'-DFUR into 5-FU, while TS interferes with DNA synthesis by causing formation of a ternary complex between 5-fluorodeoxyuridine monophosphate (F-dUMP), which is a metabolite of 5-FU, and active folic acid. DPD is an enzyme that inactivates 5-FU. Accordingly, in general, higher TP expression and lower DPD expression inside tumors result in higher intratumoral concentrations of 5-FU and a more potent antitumor effect (13-15). It is also said that the

antitumor effect is greater when expression of TS is low. Being able to quantitatively assay the expression or activity of TP, TS and DPD would have a high level of clinical significance, but fresh biological samples are necessary for quantitative assay (7, 16). Moreover, the available quantitative assays are technically complicated and thus difficult for the ordinary clinical facility to perform. In contrast, immunohistochemical staining techniques are comparatively simple. In the present study, we administered capecitabine monotherapy to Japanese women with advanced or recurrent breast cancer and in whom the clinical efficacy was able to be evaluated. In addition, surgically resected specimens were subjected to immunohistochemical staining for each of TP, TS and DPD, and the staining results were rated using a four-stage scoring system in order to yield semiquantitative results (8). Finally, the scores for those enzymes were analyzed for correlations with the clinical efficacy results.

In the results of the clinical trials on patients with recurrent breast cancer in Japan and other countries, high efficacy rates (30-40%) by capecitabine monotherapy were observed (17).

In spite of the fact that most of the patients included in this study had undergone previous treatment with an anthracycline or taxan, capecitabine monotherapy showed an ORR of 20% (5/25 patients), while the clinical benefit (PR plus long SD cases) rate was 40% (10/25 patients). It has been reported that the antitumor effect of capecitabine is potentiated by combined administration with a cytokine that induces intratumoral expression of TP or with other antitumor drugs (11, 16). Combined administration with taxan has been reported to be particularly effective in this regard (11). In addition, efficacy was observed even in patients previously treated with another 5-FU derivative. We surmise that these good results were because capecitabine is a drug that selectively increases the intratumoral 5-FU concentration to a greater extent than occurs with other 5-FU derivatives (10-12).

In this study, we analyzed the therapeutic efficacy results for correlations with the semiquantitative scores assigned to the expressions of each of TP, TS and DPD. The TP score showed a tendency to be higher in the PR and SD patients compared with the PD patients, but the differences were not statistically significant. The DPD score showed a tendency to be lower in the PR patients compared with the SD and PD patients, but again the differences were not statistically significant. These correlations between the expressions of TP and DPD and the therapeutic response are in agreement with the results reported for the use of 5'-DFUR as postoperative adjuvant therapy in breast cancer and colon cancer patients (5, 14, 18, 19). With regard to TS expression, it was reported that the clinical efficacy of 5-FU was good in pancreatic cancer patients who showed low expression of TS (20), and it was also reported that TS is an important prognostic factor in other malignancies, such as colon cancer (18, 21, 22). However, our present study found no correlation between TS expression and the clinical response of breast cancer. In addition, we were also able to analyze the relationships of the combinations of TP/DPD, TP/TS and TS/DPD to the clinical response. Because we thought that a clearer result may be provided by comparing the ratio of the each TP, DPD and TS. TP/DPD showed a significant correlation, and TP/TS also showed a correlation although it was not statistically significant. These findings are in agreement with the reported results for studies at the cellular level that the combination of TP/DPD showed a higher correlation with the therapeutic effect than either TP or DPD alone (12). The number of patients in our study was small, but our findings show that immunohistochemical staining of patient surgical specimens to elucidate the expressions of TP, TS and DPD indicate the possibility of individualization of chemotherapy employing capecitabine. Further studies of a larger number of patients are needed to establish the clinical significance of this approach, and studies are also warranted with regard to the effectiveness of induction of TP expression by use of cytokines, anticancer drugs and other drugs such as gefitinib (11, 16, 22, 23). The results of such studies can be thought to contribute to further improvement of the efficacy of capecitabine.

CONCLUSION

Capecitabine monotherapy was effective in the treatment of patients with advanced or recurrent breast cancer. The expression score for the combination of TP/DPD showed a statistically significant correlation with the clinical response, and the expression score for TP/TS also showed a correlation although it was not statistically significant. These findings indicate the potential for individualization of the therapeutic approach.

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