Positive Correlation between the Efficacy of Capecitabine and Doxifluridine and the Ratio of Thymidine Phosphorylase to Dihydropyrimidine Dehydrogenase Activities in Tumors in Human Cancer Xenografts

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ABSTRACT

Capecitabine (N4-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine) is a new fluoropyrimidine carbamate, which is converted to 5-fluorouracil (5-FUra) selectively in tumors through the intermediate metabolite 5'-deoxy-5-fluorouridine (5'-dFUrd, doxifluridine). 5'-dFUrd is metabolized to 5-FUra by thymidine phosphorylase (dThdPase) located in high levels in various types of solid tumors from patients, whereas 5-FUra generated is catabolized to dihydrofluorouracil by dihydropyrimidine dehydrogenase (DPD). The present study investigated whether the efficacy of capecitabine and its intermediate metabolite 5'-dFUrd correlates with levels of these enzymes in various human cancer xenograft models. Capecitabine and 5'-dFUrd were highly effective and inhibited tumor growth by more than 50% in 18 of 24 xenograft lines (75%) and 15 of 24 xenograft lines (63%), respectively, whereas 5-FUra and a mixture of tegafur and uracil were effective only in 1 of 24 (4.2%) and 5 of 24 (21%), respectively. The efficacy of capecitabine correlated with dThdPase activity. However, capecitabine was effective even in tumors with lower levels of dThdPase if DPD levels were very high. The efficacy of capecitabine consequently correlated very well with and depended on the ratio of these two enzymes in tumors. These results indicate that capecitabine might exert its efficacy through 5-FUra generated in tumor tissues but not through that generated in normal organs. On the other hand, there was no correlation between the efficacy of a mixture of tegafur and uracil and these enzyme activities in tumors. The efficacy of capecitabine would be optimized by selecting patients who have tumors with a high ratio of dThdPase to DPD activities.

INTRODUCTION

It would be helpful in cancer chemotherapy if we could predict the efficacy of a compound before treatment starts. A number of methods for predicting the susceptibility of tumors to cytostatics have been investigated. Methods such as direct antiproliferation assays in athymic mice bearing tumors excised from patients (1, 2), human tumor clonogenic assay (3), and subrenal capsule assay (4) have been examined. Studies on predictive factors in tumor tissues have also been studied, such as oncogene (5-7) and hormonal receptor status (8, 9). For prodrugs, levels of the enzymes essential for their activation can be predicted by measuring levels of the enzymes increasing and correlated with the efficacy of Capecitabine and discuss whether levels of these enzymes could be used as predictive factors for capecitabine.

MATERIALS AND METHODS

Animals. Male and female BALB/c- nu/nu mice were obtained from SLC Inc. (Hamamatsu, Japan). After at least 1 wk of observation, the mice were used at the age of 6-7 wk.

Tumors. The human cancer lines used were obtained from the following institutions: colon cancer CFPX20, gastric cancer GEXF97, and breast cancer MAXF401 from Prof. H. H. Fiebig (Freiburg University, Freiburg, Germany); gastric cancer MKN45 and MKN28 from Immunobiochemical Laboratories (Fujiko, Japan); breast cancer MCF-7 from Dr. Y. lino (Gunma University, Maebashi, Japan); breast cancer MX-1 from Dr. T. Tashiro (Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan); cervix cancer Yumoto from Dr. H. Tokita (Chiba Cancer Center, Chiba, Japan); bladder cancer T-24 from Dr. H. Akaza (Tsukuba University, Tsukuba, Japan); ovarian cancer Nakajima from Dr. S. Adachi (Niigata University, Niigata, Japan); and the other cancer line cells were maintained in \textit{in vitro} cultures. Human Cancer Xenograft Models. Small pieces of human cancers maintained by \textit{in vivo} passages were inoculated s.c. into mice with a trochar. A single-cell suspension (0.5-1 \times 10^7 cells per mouse) of estrogen receptor-positive breast cancers, ZR-75-1 and MCF-7, was inoculated into the mammary fat pad of mice implanted with a 60-day release pellet of 17ß-estradiol (0.25 mg; Innovative Research, Rockville, MD). The other cancer cell lines maintained by \textit{in vitro} cultures were inoculated s.c. into mice. To evaluate the antitumor effect of the fluoropyrimidines, tumor size and body weight were measured twice a week. The tumor volume was estimated by using the following equation: \(V = \frac{ab^2}{2}\), where \(a\) and \(b\) are tumor length and width, respectively.

Cytostatics. 5-FUra and UFT were purchased from Kyowa Hakko (Tokyo, Japan) and Taiho Pharmaceutical Co. (Tokyo, Japan), respectively. Doses of UFT are expressed as those of tegafur. 5'-dFUrd (doxifluridine) was obtained from Hoffmann-La Roche, Inc. (Basel, Switzerland), whereas capecitabine was synthesized by a method described elsewhere. All of the compounds were dissolved or suspended in 40 mM citrate buffer (pH 6.0) containing 5% gum arabic as the vehicle and were administered by the p.o. route. These four...
fluoropyrimidines to be tested were administered daily for 5 or 7 days/wk for 2-4 wk.

**dThdPase Assay.** Tumor tissues were homogenized in 10 mM Tris buffer (pH 7.4) containing 15 mM NaCl, 1.5 mM MgCl₂, and 50 μM potassium phosphate. The homogenate was then centrifuged at 105,000 X g for 90 min. The supernatant was dialyzed overnight against 20 mM potassium phosphate buffer (pH 7.4) containing 1 mM β-mercaptoethanol and used as a source of crude enzyme. The protein concentration was determined by the methods of Lowry et al. (14). All procedures were carried out below 4°C. The reaction mixture (120 μl) for the assay of the enzyme activity contained 183 mM potassium phosphate (pH 7.4), 10 mM 5'-dFUrd, and the crude enzyme from tumor tissues. The reaction was carried out at 37°C for 60 min and then terminated by the addition of 360 μl of methanol. After removal of the precipitate by centrifugation, an aliquot of the reaction mixture (100 μl) was added with 20 μM 5-chlorouracil as the internal standard and then applied to the HPLC column (ERC-ODS-1171). The solvent system used was as follows: 50 mM phosphate buffer (pH 6.8) containing 5 mM 1-decane sulfonic acid/methanol (85:15, v/v). The amount of 5-FUra produced was measured with a UV monitor (280 nm). dThdPase activity was expressed as μg of 5-FUra converted/mg of protein/h.

**DPD Assay.** The enzyme activity was determined by measuring the sum of the 5-FUra catabolites, such as FUH₂, a-fluoro-ß-ureidopropionate, and a-fluoro-ß-alanine, formed from [6-14C]5-FUra. The standard reaction mixture (50 μl) contained 10 mM potassium phosphate (pH 8.0), 0.5 mM EDTA, 0.5 mM β-mercaptoethanol, 1 mM DTT, 2.5 mM MgCl₂, 250 μM NADPH, 25 μM [6-14C]5-FUra (56 μCi/mmole), and crude enzyme (final protein concentration, 1-10 mg/ml). The reaction was carried out at 37°C for 30 min and then terminated by immersing the reaction tubes in a boiling water bath for 1 min. The reaction tubes were frozen at −20°C for at least 20 min before any further manipulations were undertaken. Proteins were removed by centrifugation, and then 10 μl of the supernatant fluid were spotted on silica gel TLC sheets (Merck 5735), which were prepsotted with 5 μl of an authentic markers mixture of 10 mM 5-FUra, 50 mM dihydrouracil, 20 mM β-ureidopropionate, 10 mM a-fluoro-ß-alanine, and 50 mM urea. The spots were developed in a solvent system of a mixture of ethylacetate/isopropanol/H₂O (65:23:12, v/v/v). These developed markers were identified by the methods of Naguib et al. (13). Spots identified as the 5-FUra catabolites were cut out, and then their radioactivities were counted in a scintillation counter. DPD activity was expressed as pmol 5-FUra converted/mg of protein/min.

**Statistical Analysis.** Tumor size and enzyme activities were analyzed using the ANOVA test and Mann-Whitney U test. Differences were considered to be significant at P < 0.05.

**RESULTS**

**Antitumor Efficacy.** The antitumor activities of capecitabine and other fluoropyrimidines given p.o. were compared in 24 human cancer xenograft models, which included colon, gastric, breast, cervical, bladder, ovarian, and prostate cancer models. Capecitabine, 5'-dFUrd, 5-FUra, and UFT were given for 10-28 days to mice bearing the xenografts at their maximum tolerated doses, which were determined with the HCT116 xenograft model (Table 1); they were 10.5, 5.25, 1.05, and 0.7 mmol/kg/wk (p.o., every day for 5 or 7 days/wk), respectively. Table 2 summarizes the study results that the efficacy of capecitabine was superior to that of 5'-dFUrd, 5-FUra, and UFT in many xenograft models. Capecitabine was effective (defined as >50% growth inhibition) in 18 of 24 xenograft models (75%) and inhibited tumor growth by more than 90% in 7 of 24 models. In contrast, 5'-dFUrd showed more than 90% tumor growth inhibition in only 1 of 24 models, although it was effective in 15 of 24 models (63%). 5-FUra and UFT were effective only in 1 of 24 (4.1%) and 5 of 24 (21%) models, respectively, and inhibited the growth by no more than 90%.

**Enzyme Activities in Tumors.** The activities of dThdPase and DPD, which might affect 5-FUra levels, in each cancer xenograft were measured. These two enzyme activities (Figs. 1 and 2) and their ratio (Fig. 3) varied from one xenograft to another. A correlation of the enzyme levels with susceptibility of the xenograft to capecitabine, 5'-dFUrd, and UFT, was examined. The susceptibility to capecitabine appears to be correlated with dThdPase levels in tumors and inversely correlated with DPD levels to a slight extent (Fig. 4A). Namely, in many of the susceptible xenograft lines, dThdPase activity is higher, and DPD activity is lower than in refractory lines. However, there are some discrepancies between the susceptibility to capecitabine and either enzyme level in tumors. Some lines with low levels of dThdPase were susceptible because of low DPD activity (MKN45 and COLO205), and other lines with high levels of DPD were also susceptible because of high dThdPase activity (HT-3 and Scaber). Therefore, the susceptibility correlates with the ratio of these two enzyme activities in tumors to a greater extent. The susceptibility of the tumor to 5'-dFUrd showed similar patterns of correlation with the enzyme activities (Fig. 4B). In contrast, susceptibility of the xenograft to UFT and the levels of these two enzyme activities did not correlate well (Fig. 4C).

**DISCUSSION**

The new cytostatic agent capecitabine generates the active drug 5-FUra from its intermediate metabolite 5'-dFUrd by dThdPase (10, 11), whereas 5-FUra is catabolized to FUH₂ by DPD (12, 13). The present study showed that the efficacy of capecitabine and 5'-dFUrd correlated with these enzymes in tumors in human cancer xenograft models, particularly with the ratio of these enzymes in tumors. These results indicate that the conversion of 5'-dFUrd to 5-FUra in tumor tissues should be essential for the efficacy of capecitabine and support our previous observation that capecitabine is converted to 5-FUra selectively in tumor tissues (15). The tumor-selective conversion to 5-FUra would be a result of unique localization of the enzyme dThdPase, which exists highly in tumor tissues in humans (10, 11) and now is known as an angiogenic factor (16, 17). The tumor-selective conversion explains the higher antitumor activity and broader antitumor spectrum of capecitabine, compared with those of the administration of 5-FUra itself.

The efficacy of UFT, a fixed combination drug, did not correlate with tumor levels of DPD in the present study, although UFT is reported to exert its efficacy through 5-FUra produced. Tegafur is mainly converted to 5-FUra by the P450 drug-metabolizing enzyme in the liver (18), and the 5-FUra is distributed to tumors and normal tissues through the circulation. Uracil, a component of UFT, would
**EFFICACY OF CAPECITABINE CORRELATES WITH dThdPase AND DPD**

Table 2 Antitumor activity of four fluoropyrimidines against human cancer xenografts

<table>
<thead>
<tr>
<th>Cancer origin</th>
<th>Cancer xenograft</th>
<th>Treatment schedule</th>
<th>Vehicle</th>
<th>Capecitabine</th>
<th>5'-dFUr</th>
<th>5'-FUra</th>
<th>UFT</th>
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<tr>
<td>Colon</td>
<td>CFP280</td>
<td>×15 (qd ×5/wk)</td>
<td>913</td>
<td>36 (96)</td>
<td>211 (77)</td>
<td>393 (57)</td>
<td>314 (66)</td>
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<td>HCT116</td>
<td>×15 (qd ×5/wk)</td>
<td>1188</td>
<td>−9 (101)</td>
<td>327 (72)</td>
<td>728 (39)</td>
<td>795 (33)</td>
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<td>LoVo</td>
<td>×20 (qd ×5/wk)</td>
<td>521</td>
<td>115 (78)</td>
<td>202 (61)</td>
<td>433 (17)</td>
<td>250 (52)</td>
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<td></td>
<td>COLO205</td>
<td>×20 (qd ×5/wk)</td>
<td>1259</td>
<td>495 (61)</td>
<td>586 (53)</td>
<td>813 (35)</td>
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<td></td>
<td>HT-29</td>
<td>×20 (qd ×5/wk)</td>
<td>745</td>
<td>488 (34)</td>
<td>580 (22)</td>
<td>543 (27)</td>
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<td></td>
<td>DLD-1</td>
<td>×10 (qd ×5/wk)</td>
<td>876</td>
<td>640 (27)</td>
<td>545 (38)</td>
<td>699 (20)</td>
<td>492 (44)</td>
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<td></td>
<td>WiDr</td>
<td>×21 (qd ×7/wk)</td>
<td>1373</td>
<td>745 (46)</td>
<td>881 (36)</td>
<td>907 (34)</td>
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<td>Gastric</td>
<td>GXF97</td>
<td>×21 (qd ×7/wk)</td>
<td>613</td>
<td>40 (94)</td>
<td>185 (70)</td>
<td>352 (43)</td>
<td>251 (59)</td>
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<td>MKN45</td>
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<td>881</td>
<td>−16 (102)</td>
<td>306 (65)</td>
<td>630 (28)</td>
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<td>MKN28</td>
<td>×20 (qd ×5/wk)</td>
<td>1252</td>
<td>353 (72)</td>
<td>768 (39)</td>
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<td>Breast</td>
<td>ZR-75-1</td>
<td>×15 (qd ×5/wk)</td>
<td>406</td>
<td>−20 (105)</td>
<td>44 (89)</td>
<td>363 (11)</td>
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<td>MCF-7</td>
<td>×15 (qd ×5/wk)</td>
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<td>257 (72)</td>
<td>848 (9)</td>
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<td>1571</td>
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<td>1406 (11)</td>
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<td>Yumoto</td>
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<td>37 (98)</td>
<td>60 (96)</td>
<td>1320 (20)</td>
<td>1243 (24)</td>
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<td></td>
<td>ME-180</td>
<td>×15 (qd ×5/wk)</td>
<td>479</td>
<td>99 (79)</td>
<td>228 (52)</td>
<td>434 (9)</td>
<td>442 (8)</td>
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<td></td>
<td>SIHA</td>
<td>×20 (qd ×5/wk)</td>
<td>1200</td>
<td>381 (68)</td>
<td>462 (62)</td>
<td>1171 (2)</td>
<td>887 (25)</td>
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<td></td>
<td>HT-3</td>
<td>×21 (qd ×7/wk)</td>
<td>1496</td>
<td>511 (66)</td>
<td>931 (38)</td>
<td>1301 (13)</td>
<td>1366 (9)</td>
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<td>Bladder</td>
<td>Scaber</td>
<td>×28 (qd ×7/wk)</td>
<td>997</td>
<td>50 (95)</td>
<td>483 (52)</td>
<td>687 (31)</td>
<td>192 (81)</td>
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<td></td>
<td>T-24</td>
<td>×10 (qd ×5/wk)</td>
<td>437</td>
<td>415 (3)</td>
<td>390 (11)</td>
<td>434 (1)</td>
<td>361 (18)</td>
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<td>×16 (qd ×7/wk)</td>
<td>944</td>
<td>52 (89)</td>
<td>90 (82)</td>
<td>572 (16)</td>
<td>294 (40)</td>
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<td>Ovary</td>
<td>SK-OV-3</td>
<td>×28 (qd ×7/wk)</td>
<td>2632</td>
<td>2909 (−11)</td>
<td>2516 (4)</td>
<td>2508 (5)</td>
<td>3200 (−22)</td>
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<td>Prostate</td>
<td>PC-3</td>
<td>×20 (qd ×5/wk)</td>
<td>627</td>
<td>146 (77)</td>
<td>367 (42)</td>
<td>389 (38)</td>
<td>364 (42)</td>
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* Susceptibility based on more than 50% tumor growth inhibition.

also distributed to tumors and normal tissues with pharmacokinetic profiles different from those of tegafur and would competitively inhibit 5-FUra degradation by DPD (19). It is therefore unlikely that the DPD activity in tumor tissue extracts studied (dialyzed samples) is comparable to that in tumors under UFT administration. The efficacy of capecitabine also did not significantly correlate with tumor levels of DPD, unlike 5'-dFUrd. This was because of the difference in the susceptibility to 5'-dFUrd and capecitabine of one particular xenograft line, HT-3, which has the highest DPD activity among the xenograft lines studied. HT-3 is classified as a line refractory to 5'-dFUrd, whereas it is susceptible to capecitabine probably because it could be given safely at higher doses than those of 5'-dFUrd. We think that the dThdPase:DPD ratio in tumors correlates with the susceptibility to 5'-dFUrd and capecitabine to a greater extent than either DPD activity or DPD activity alone.

The efficacy of capecitabine and 5'-dFUrd correlated well with the ratio of dThdPase:DPD in tumor tissues. If this is indeed the case in clinical trials, the efficacy of these compounds would be predicted by measuring these two enzyme levels in the tumor before treatment is begun. This would be likely, because it is suggested that measuring

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![Fig. 1. dThdPase activities in tumor tissues of 24 human cancer xenograft lines. The enzyme activity (mean ± SD (bars); n = 3) was determined as described in "Materials and Methods." The susceptibilities of human cancer xenografts to each fluoropyrimidine were expressed as +, susceptible, >50% tumor growth inhibition; and −, refractory, <50% tumor growth inhibition. Details of these susceptibilities are shown in Table 2.](image-url)
EFFICACY OF CAPECITABINE CORRELATES WITH dThdPase AND DPD

even only dThdPase would be useful as a predictive factor in the subsequent adjuvant chemotherapy with 5'-dFUrd (doxifluridine) (20), which is now prescribed for the treatment of breast, colorectal, gastric, and other cancers in Japan, Korea, and China and is now being assessed in Europe. Yamamoto et al. (21) reported that advanced breast cancer patients with a dThdPase-positive primary tumor responded well to 5'-dFUrd. Tumor dThdPase and DPD activity-driven chemotherapy would avoid unnecessary treatment with capecitabine and 5'-dFUrd and would increase the response rate to these compounds.

Cancer chemotherapy would be helped if we could predict the efficacy of a drug before treatment is begun. A number of studies on
methods for predicting the susceptibility of cancers to cytostatics have been conducted, such as those in athymic mice bearing tumors excised from patients (1, 2), human tumor clonogenic assay (3), and subrenal capsule assay (4). In addition, various factors related to tumors are reported to correlate with the efficacy of anticancer drugs, such as thymidylate synthetase status for 5-FUra (22) and p53 status for some anticancer drugs (5). However, no useful methods to date have been identified except for that measuring estrogen receptor status for tamoxifen (9). The present study indicates that the efficacy of capecitabine and its intermediate metabolite 5′-dFUrd (dofluridine) may be predicted by measuring the levels of dThdPase and DPD in tumor tissues. These enzyme assays and subsequent treatment with the cytostatics could constitute a new treatment modality, tumor enzyme activity-driven chemotherapy. Clinical studies using this approach for predicting the efficacy of capecitabine and 5′-dFUrd would be warranted.

We reported previously that dThdPase is up-regulated by several factors, such as interleukin 1α, tumor necrosis factor α, and IFNγ.
(23), and indirectly by interleukin 12 (24) and taxol (25). Consequently, these factors enhanced the antiproliferative activity of the in vivo antitumor activity of 5′-dFdUrd. On the other hand, DPD inhibitors modulate the efficacy of fluoropyrimidines. The DPD inhibitors 5-ethynyluracil and 5-chloro-2,4-dihydroxypyrimidine were reported to enhance the efficacy of 5-FUra (26) and tegafur (27), respectively. Combination with capecitabine therapy and dThdPase and DPD modulators would be one rational approach, and it would be worthwhile to pursue future studies. However, dThdPase and DPD modulators may also have the potential of increasing the toxicity of capecitabine. We will further investigate methods optimizing the efficacy of capecitabine and thereby increasing its value as a therapeutic agent.

REFERENCES

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