Schedule Dependency of Antitumor Activity in Combination Therapy with Capecitabine/5'-Deoxy-5-fluorouridine and Docetaxel in Breast Cancer Models

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ABSTRACT

Docetaxel and capecitabine are being prescribed for the treatment of breast cancer. In this study, we tried to identify the optimal administration schedule in combination therapy with these anticancer drugs in human cancer xenograft models. Capecitabine was given p.o. daily for 2 weeks (days 1–14), whereas docetaxel was given i.v. on day 1, day 8, or day 15 in a 3-week regimen to the mice bearing MX-1 human breast cancer xenograft. The combination showed better antitumor efficacy than the monotherapy of either agent in either dosing regimen. However, the most potent and synergistic activity was observed when docetaxel was given on day 8. This potent effect appears to be characteristic of the combination of docetaxel with capecitabine or its intermediate metabolite 5'-deoxy-5-fluorouridine (doxifluridine; 5'-dFUrd). Docetaxel given on day 8 showed a potent effect in combination with 5'-dFUrd, but a much weaker effect was observed in combination with 5-fluorouracil or UFT, a fixed combination of tegafur and uracil. Better efficacy was also observed in the MAXF401 human breast cancer xenograft and in the mouse A755 mammary tumor when docetaxel was given at the middle of the capecitabine or 5'-dFUrd treatment rather than other dosing regimens. In contrast, the efficacy in WiDr human colon cancer xenograft was somewhat better when docetaxel was given on day 1. One possible explanation for the synergy is that docetaxel up-regulates tumor levels of thymidine phosphorylase, the enzyme essential for the activation of capecitabine or 5'-dFUrd to 5-fluorouracil. In fact, docetaxel up-regulated the thymidine phosphorylase levels 4.8- and 1.9-fold in the WiDr and MX-1 models, respectively. However, it did not significantly up-regulate in the MAXF401 and A755 models in which a potent combination effect was observed as well. Other mechanisms, particularly those for the synergy with docetaxel given at the middle during capecitabine/5'-dFUrd administration, would also exist. Based on these observations, clinical studies on the day 8 combination regimen with docetaxel and capecitabine/5'-dFUrd are warranted.

INTRODUCTION

In the treatment of solid cancers, a combination of anticancer agents often increases the number of complete responses and the duration of the responses (1). Establishment of an optimal regimen for combination therapies with newly developed drugs is an important step to achieve higher response and longer survival. Taxanes and fluoropyrimidines are of a different class of agents; both are effective in breast cancer therapy, and hence efficacy in combination is anticipated. Although paclitaxel and 5-FUra2 in combination was reported to be subadditive or antagonistic in in vitro experiments (2–4), in vivo efficacy in combination with taxanes and fluoropyrimidine prodrugs, capecitabine and 5'-dFUrd, has been reported to be more than additive (5). In the present study, we have investigated an optimal administration schedule in combination with docetaxel and fluoropyrimidines, such as capecitabine and 5'-dFUrd, in mammary tumor models.

Capecitabine (N9-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine; Xeloda) is a fluoropyrimidine carbamate that is being used clinically in an oral form for the treatment of breast cancer patients who have failed paclitaxel and anthracycline regimens (6). It is also being assessed for the treatment of other types of cancer, including colorectal, gastric, and pancreatic. It generates 5-FUra selectively in tumors through three enzymes present in the liver, then to 5'-dFUrd (doxifluridine; Furtulon) by cytidine deaminase with high activity in the liver and in various solid tumors, and finally to 5-FUra by dThdPase with high activity in many types of tumors (7). An oral form of intermediate metabolite 5'-dFUrd is being prescribed in Japan, Korea, and China for the treatment of breast, colorectal, gastric, bladder, and cervical cancers (9). Another fluoropyrimidine, UFT, is a mixture of tegafur, a prodrug of 5-FUra, and uracil. Tegafur is converted to 5-FUra mainly by hepatic P450 (10).

The examination of docetaxel and capecitabine/5'-dFUrd

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in combination is of interest for efficacy in the treatment of breast cancer, because the two have different modes of action and different toxicity profiles. Furthermore, in a previous study Sawada et al. demonstrated in human tumor xenograft models that taxanes have the ability to up-regulate tumor levels of dThdPase, an enzyme essential for the activation of capecitabine and 5'-dFUrd (5). In the present study, to obtain insight into optimal dosing regimens for clinical studies, we have compared different administration schedule with docetaxel and capecitabine/5'-dFUrd in combination in mammary tumor models. The results showed that there is a characteristic schedule dependency in antitumor activity. The most effective administration was docetaxel injection in the middle of two weeks of daily oral administration of capecitabine or 5'-dFUrd. The possible mechanism behind this observed schedule dependency is discussed herein.

MATERIALS AND METHODS

Animals. Five-week-old BALB/c-nu/nu and 4-week-old female C57BL/6 mice were obtained from Japan SLC, Inc. (Hamamatsu, Japan) or Charles River Japan, Inc. (Yokohama, Japan). They were kept for 1 week in our animal facility before tumor inoculation.

Tumors. The tumor lines used were provided by the following institutions: human mammary tumor MX-1 (11) from Dr. T. Tashiro (Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan); human mammary tumor MAXF401 (12) from Prof. H. H. Fiebig (University of Freiburg, Freiburg, Germany); murine mammary tumor A755 (13) from Dr. M. Igo (National Cancer Center Research Institute, Tokyo, Japan); human colon tumor WiDr from American Type Culture Collection (Manassas, VA). MX-1 and MAXF401 were maintained by in vivo passages in female BALB/c-nu/nu mice, whereas A755 was maintained in C57BL/6 mice. WiDr was maintained in tissue culture.

Human Cancer Xenograft and Murine Tumor Model. A small piece of MX-1 or MAXF401 was transplanted s.c. into female nude mice. A755 tumor tissues were minced and passed through a wire mesh. A suspension of A755 tumor cells (3 × 10^5 viable cells/mouse) was inoculated s.c. into female C57BL/6 mice. A suspension of WiDr tumor cells (5 × 10^6 viable cells/mouse) was inoculated s.c. into male nude mice. The experiments for human tumor xenografts and A755 were started when the tumor volumes reached ~0.3–0.5 and 1.2 cm^3, respectively. The numbers of animals in each experiment group are specified in the legend of each table and figure. The tumor volumes were estimated by using the equation, \[ V = \frac{ab^2}{2}, \] where \( a \) and \( b \) are tumor length and width, respectively. To evaluate the antitumor effect of the fluoropyrimidines and docetaxel, tumor sizes and body weights were measured two or three times a week. Carcass body weight was calculated by subtracting the tumor weight, which was estimated from tumor volume, from the body weight. Bone marrow toxicity was estimated by counting peripheral blood leukocytes. Gastrointestinal toxicity was estimated by observing the feces and by detecting occult blood in the feces using a test kit (Shionogi Pharma Co., Osaka, Japan). All animal experiments were conducted in accordance with the “Guidelines for the Care and Use of Laboratory Animals in Nippon Roche Research Center.”

Chemicals. Capecitabine was synthesized by the method described elsewhere (14). 5'-dFUrd was synthesized at Hoffmann-La Roche (Basle, Switzerland). Docetaxel (Taxotere) was...
RESULTS

Optimal Administration Schedule for Combination Therapy with Docetaxel and Capecitabine in Human Mammary Tumor Xenograft MX-1 Model. The standard clinical dosing regimen for docetaxel is one i.v. infusion every 3 weeks and for capecitabine 2 weeks of daily oral administration in 3-week cycles. We first compared antitumor activity of three typical regimens in combination using a human mammary tumor xenograft MX-1 model. Capecitabine was administered p.o. for 14 days (days 1 to 14), whereas docetaxel was injected once on (a) the first day of capecitabine administration (d1 combination); (b) in the middle (day 8) of capecitabine administration (d8 combination), or (c) on the day after the capecitabine administration (day 15 combination). The dose of capecitabine used, 359 mg/kg, is two-thirds of its MTD (8). The dose of docetaxel used, 7.5 mg/kg, is 1/15 of its highest nonlethal dose (16). As MX-1 is highly sensitive to docetaxel treatment (16), we chose a low dose of docetaxel to demonstrate schedule dependency of the combination therapy. In a separate experiment, we also examined the dose response of capecitabine and docetaxel as single agents. Capecitabine in doses of 180, 359, and 539 mg/kg induced 36, 57, and 65% inhibition of tumor growth, respectively, at the end of a 3-week cycle (day 22). Docetaxel injection on day 8 in doses of 5, 7.5, 10, and 15 mg/kg induced 9, 41, 67, and 81% inhibition of tumor growth, respectively, on day 22.

As shown in Fig. 1, either capecitabine or docetaxel as monotherapy showed only moderate antitumor activity at these suboptimal doses for each agent. In contrast, combination therapy in every regimen showed better antitumor activity than monotherapy of either agent. Especially, the day 8 combination showed the most potent activity among the three regimens, and it appears to be more than just additive. In this day 8 combination group, tumors in three of eight mice were completely regressed at 2 weeks after termination of the capecitabine treatment. None of the treatment groups in this experiment showed carcass weight loss due to drug toxicity. Using the data of single agent dose responses and the day 8 combination, we performed isobologram analysis. The result revealed that capecitabine and docetaxel in combination exhibited a synergistic effect (data not shown).

In another experiment, we compared the timing of docetaxel injections in detail. Docetaxel was injected either day 1, day 3, day 6, day 8, day 10, day 13, or day 15 in combination with 2 weeks of daily p.o. administration of capecitabine. The results showed that there was clear schedule dependency in terms of the maximum inhibitory rate of tumor growth (fold reduction), and the most active regimen was the day 10 combi-
Activity of Capecitabine and Docetaxel in Combination

We next examined the efficacy of another combination, Adriamycin-cyclophosphamide (AC) and docetaxel, in different dosing regimens as a control experiment. When AC were injected i.p. into the mice bearing MX-1, tumor growth to a volume of 2000 mm³ was delayed 8 days, whereas docetaxel (7.5 mg/kg) injected i.v. resulted in 4 days of delay. As combination groups, three timings of injections were compared: docetaxel on day 1 and AC on day 8; both docetaxel and AC on day 1; and AC on day 1 and docetaxel on day 8. These regimens induced growth delays of 12, 14, and 12 days, respectively; namely, every regimen of docetaxel and AC in combination induced only additive growth delay.

Thus, observed potent antitumor activity in combination is a unique feature of capecitabine/5'-dFURd and docetaxel combination.

Optimal Dosing Regimen for the Combination Therapy in Other Mammary Tumor Models. We further compared efficacy of different regimens of docetaxel combinations with 5'-dFURd in other tumor models: human mammary tumor MAXF401; murine mammary tumor A755 and human colon tu-

Fig. 3  Antitumor activity of repeated combination regimens with capecitabine and docetaxel on tumor growth of MX-1 human mammary tumor xenografts. Mice bearing MX-1 tumors were randomized into groups of six mice each. Three weeks of combination regimens described in Fig. 1 were repeated twice beginning 16 days after the tumor inoculation. Namely, capecitabine (359 mg/kg) was administered p.o. for 14 days twice beginning 16 and 37 days after the tumor inoculation, whereas docetaxel (7.5 mg/kg) was injected i.v. twice either on the first day of (A, d1, 22 combination), on the eighth day of (B, d8, 29 combination) or on the next day of (C, d15, 36 combination) docetaxine administration. Data are mean ± SD (vertical bars) of tumor volume. Horizontal bars, timing of capecitabine administration; arrows, timing of docetaxel injection. ⊙, vehicle; △, capecitabine alone; □, docetaxel alone; ■, capecitabine and docetaxel in combination.

Optimal Dosing Regimen for Combination Therapy

We further compared the effects of combinations on tumor growth delay of four fluoropyrimidines, capecitabine, 5'-dFURd, 5-FUra, and UFT, in the day 8 combination regimen with docetaxel (Table 1). Two doses of fluoropyrimidines, at two-thirds of the MTD and at the MTD for each drug (8, 15), were used. In the case of UFT, 1.5-fold of its MTD was also included. Tumor growth delays induced by either fluoropyrimidines or docetaxel as a single agent were rather short; all of them were within 10 days. Capecitabine or 5'-dFURd in combination with docetaxel, however, caused tumor growth delay of from 40 to >60 days, far longer than merely an additive delay. In contrast, growth delay observed in the 5-FUra or UFT combination was <30 days. Furthermore, complete regression of the tumor was observed more often in the capecitabine or 5'-dFURd combination groups than in 5-FUra or UFT combinations. Thus, the potent antitumor activity of the day 8 regimen seems to be characteristic of the combination of capecitabine and 5'-dFURd.

We also examined the efficacy of another combination, Adriamycin-cyclophosphamide (AC) and docetaxel, in different dosing regimens as a control experiment. When AC were injected i.p. into the mice bearing MX-1, tumor growth to a volume of 2000 mm³ was delayed 8 days, whereas docetaxel (7.5 mg/kg) injected i.v. resulted in 4 days of delay. As combination groups, three timings of injections were compared: docetaxel on day 1 and AC on day 8; both docetaxel and AC on day 1; and AC on day 1 and docetaxel on day 8. These regimens induced growth delays of 12, 14, and 12 days, respectively; namely, every regimen of docetaxel and AC in combination induced only additive growth delay.

Thus, observed potent antitumor activity in combination is a unique feature of capecitabine/5'-dFURd and docetaxel combination.
mor WiDr; in addition to MX-1. In the A755 model, the period of 5'-dFUrd administration was shortened to 8 days, and docetaxel was injected on day 1, day 5, or day 9, due to rapid tumor growth. Fig. 5 shows the delay in days for a tumor to grow to 1.5-fold of the volume from the day of docetaxel injection. In the MX-1 model, the growth delay found in every combination regimen was more than additive, whereas that of the day 8 regimen was the most pronounced. In the MAXF401 model, growth delay observed in the day 8 combination was much longer than just additive delay of each agent, whereas those with day 1 or day 15 combinations were additive or less than additive. The combination of capecitabine with docetaxel also showed the same schedule dependency in tumor growth delay in the MX-1 model (Fig. 1) and MAXF401 model (data not shown). In every combination group of this experiment, 90% inhibition of the tumor growth was observed on day 22 (data not shown).

dThdPase Up-Regulation by Docetaxel. The effect of docetaxel administration on tumor levels of human dThdPase in xenograft models and murine PyNPase in the case of A755, activation enzymes for 5'-dFUrd and capecitabine, was examined (Table 3). Significant 1.9- and 4.8-fold up-regulation of dThdPase was observed 7 days after docetaxel injection in MX-1 and WiDr models, respectively. In contrast, no significant up-regulation of dThdPase in MAXF401 or that of PyNPase in A755 was observed. Examination of the host organs of mice bearing MX-1 after docetaxel injection showed that there was no significant up-regulation of PyNPase activity in the liver,
spleen, kidney, large intestine, or small intestine, although there was significant up-regulation of tumor dThdPase in the same mice (data not shown).

**DISCUSSION**

In the present study, we investigated the best dosing regimen in mammary tumor models for combination therapy with docetaxel and capecitabine/5'-dFUrd. The study clearly indicated that the efficacy depends on the dosing schedule. Docetaxel given at the middle of 2 weeks of capecitabine administration (day 6, day 8, or day 10 combination regimen) showed more potent antitumor activity in the human mammary tumor xenograft MX-1 model than that given on the first day (day 1 combination) or 1 day after the 2 weeks of capecitabine treat-

![Table 1](image)
Table 2  Toxicity of three different regimens in combination with 5'-dFUr and docetaxel in MX-1 human mammary tumor xenograft

Mice bearing MX-1 were randomized into groups of four mice each, and treatment was started 21 days after tumor inoculation. MTD of 5'-dFUr (185 mg/kg) was administered p.o. for 14 days, whereas one-fourth of highest nonlethal dose of docetaxel (30 mg/kg) was injected i.v. either on day 1, day 8, or day 15. Mice were sacrificed on the days indicated, and the blood samples collected by heart puncture were used for leukocyte count.

<table>
<thead>
<tr>
<th>Administration</th>
<th>Peripheral blood leukocyte count (×10⁶ cells/ml of blood)</th>
<th>Carcass wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 8</td>
<td>Day 15</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7.45 ± 1.33</td>
<td>8.80 ± 1.09</td>
</tr>
<tr>
<td>5'-dFUr alone</td>
<td>8.36 ± 0.50 (49)</td>
<td>5.55 ± 0.21 (63)</td>
</tr>
<tr>
<td>Docetaxel alone</td>
<td>8.75 ± 3.29 (117)</td>
<td>ND</td>
</tr>
<tr>
<td>Day 1 combination</td>
<td>2.13 ± 0.57 (29)</td>
<td>4.70 ± 2.82 (53)</td>
</tr>
<tr>
<td>Day 8 combination</td>
<td>5.73 ± 2.37 (65)</td>
<td>11.53 ± 1.94 (101)</td>
</tr>
<tr>
<td>Day 15 combination</td>
<td>10.33 ± 3.40 (154)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Numbers in parentheses, percentage of treated versus vehicle. ND, not done.

Table 3  Effect of docetaxel injection on tumor levels of dThdPase in various tumor models

Nude mice bearing indicated human tumors were given i.v. injection of docetaxel. Tumors were removed 4 days later. PyNPase activity in the tumor was determined by high-performance liquid chromatography. Number of mice per group: WiDr five; other models, four.

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Dose of docetaxel (mg/kg)</th>
<th>dThdPase level (units/mg protein)</th>
<th>PyNPase level (µg 5-FUra/mg protein/h)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td></td>
<td>Vehicle</td>
<td>Docetaxel</td>
<td></td>
</tr>
<tr>
<td>MX-1</td>
<td>7.5</td>
<td>11.3 ± 2.0</td>
<td>21.5 ± 3.6</td>
<td>1.9</td>
</tr>
<tr>
<td>MAXF401</td>
<td>7.5</td>
<td>9.0 ± 0.9</td>
<td>10.8 ± 0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>WiDr</td>
<td>15</td>
<td>2.2 ± 0.5</td>
<td>10.6 ± 2.8</td>
<td>4.8</td>
</tr>
<tr>
<td>Murine A755</td>
<td>10</td>
<td>13.1 ± 8.2</td>
<td>11.2 ± 3.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Significantly different from vehicle group; P < 0.05 by Student’s t test.

ment (day 15 combination). The superiority of docetaxel injection at the middle of the capecitabine/5'-dFUr administration over the other regimens was observed in the other mammary tumor models, MAXF401 and A755, as well. In the MX-1 model, the efficacy of the combination in the day 8 regimen was more than just additive in terms of tumor volume reduction and tumor growth delay. In separate experiments, we also observed similar results with 5'-dFUr and paclitaxel in combination. Their efficacy was also more than merely additive when paclitaxel-dFUr and paclitaxel in combination.

One possible mechanism of the synergy in vivo is that docetaxel up-regulates dThdPase, the enzyme essential for the activation of 5'-dFUr to 5-FUra, and consequently enhances the efficacy of 5'-dFUr and its prodrug capecitabine, but not the efficacy of 5-FUra or UFT, in human tumor xenograft models (5). In fact, the effect of the combination with either 5-FUra or UFT, for the efficacy of which dThdPase is not essential (15, 18), was weak in the present study. In addition, we observed that docetaxel significantly up-regulated dThdPase in mammary tumor MX-1 and colon tumor WiDr xenografts and that the efficacy of these drugs was indeed more than additive, similarly observed by Sawada et al. (5).

However, there should exist additional mechanisms of synergy with docetaxel and capecitabine/5'-dFUr, particularly of that in the day 8 regimen in the mammary tumor xenograft models. If the up-regulation of dThdPase was solely responsible for the synergy, the day 1 combination would be more effective than or equally effective as the day 8 combination, because the dThdPase up-regulation reached a peak at 6 days after docetaxel injection in human cancer xenografts (5). The present results showed that the antitumor activity observed in the day 6 through day 10 regimen was, however, more potent than that observed in the day 1 through day 3 regimen in the three mammary tumor models studied. Furthermore, docetaxel given at the middle of capecitabine/5'-dFUr administration showed a potent effect in the MAXF401 and A755 models, in which the dThdPase/PyNPase up-regulation by docetaxel was not observed. Capecitabine
administration for certain periods preceding the docetaxel injection appears to be necessary to optimize the efficacy of docetaxel in the MX-1 model, although such a mechanism has not yet been elucidated. We also observed that the efficacy of 5-FUra and docetaxel in the day 8 regimen was also somewhat better than other regimens, although the degree of the effect of the combination was much smaller than that observed with capecitabine/5'-dFUrd and docetaxel. 5-FUra may indirectly enhance the efficacy of docetaxel in vivo by unknown mechanisms and capecitabine/5'-dFUrd, which gives higher levels of 5-FUra in tumor tissues than does 5-FUra (8), may greatly enhance the efficacy of docetaxel.

We have no direct evidence supporting the idea that capecitabine/5'-dFUrd or fluoropyrimidines given at the day 8 regimen enhances the efficacy of docetaxel. However, it is reported that 5'-dFUrd given in a neoadjuvant setting in breast cancer patients reduced Bcl-2 expression and increased Bax expression in tumor tissues (20). On the other hand, it is reported that overexpression of Bcl-2 in mammary tumor cell lines made the cells resistant to paclitaxel-induced apoptosis (21), whereas the suppression of Bcl-2 expression by antisense oligodeoxynucleotides augmented antitumor activity of paclitaxel (22). Capecitabine/5'-dFUrd and 5-FUra may enhance the efficacy of taxanes through modulating tumor levels of Bcl-2 and Bax family proteins, or through other unknown mechanisms.

The dosages used in the present study for capecitabine/5'-dFUrd and docetaxel are in the ranges of their clinical studies in terms of doses per body surface area (23); capecitabine at 359 mg/kg corresponds to 1662 mg/m² in humans, 5'-dFUrd at 123 mg/kg corresponds to 569 mg/m² in humans, and docetaxel at 7.5–15 mg/kg corresponds to 35–70 mg/m² in humans. In combination at these dosages, they showed more than merely additive efficacy without greatly enhanced toxicity and were the most effective in the day 8 regimen, where docetaxel was given at the middle of the 2-week capecitabine/5'-dFUrd administration. Although the mechanism of this combination effect has not yet been clarified, clinical studies with capecitabine/5'-dFUrd and docetaxel in combination in the day 8 regimen is warranted.

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