# 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 and 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-FUra[2] 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 ($N^4$-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 liver and in tumors (7, 8). In humans, it is sequentially converted first to 5′-deoxy-5-fluorocytidine by carboxylesterase located in the liver, then to 5′-dFUrd (doxifluridine; Furtulon) by cytidine deaminase also 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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[2] The abbreviations used are: 5-FUra, 5-fluorouracil; 5′-dFUrd, 5′-deoxy-5-fluorouridine; dThdPase, thymidine phosphorylase; UFT, uracil-5-fluorouracil-tegafur; MTD, maximum tolerated dose; PyNPase, pyrimidine nucleoside phosphorylase; A/C, Adriamycin (6 mg/kg) and cyclophosphamide (60 mg/kg).

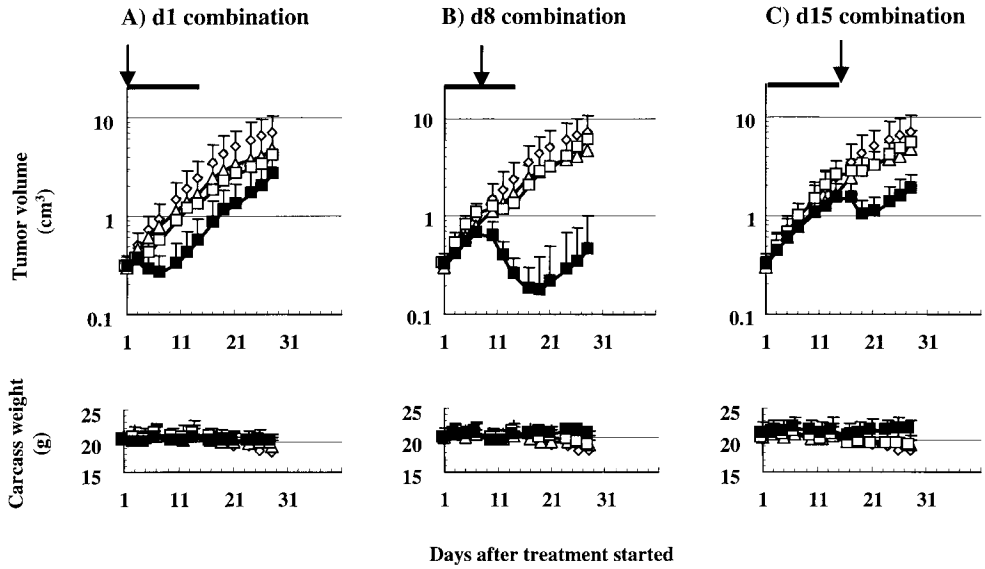


*Fig. 1* Antitumor activity of three different regimens in combination with capecitabine and docetaxel in MX-1 human mammary tumor xenografts. Mice bearing MX-1 tumors were randomized into groups of eight mice each. Capecitabine was administered p.o. for 14 days at 359 mg/kg beginning 18 days after the tumor inoculation, whereas docetaxel (7.5 mg/kg) was injected i.v. on the first day [*A*, day 1 (*d1 combination*), on the eighth day (*B, d8 combination*), or on the next day (*C, d15 combination*) of capecitabine administration. Data are mean ± SD (*vertical bars*) of tumor volume and carcass weight. *Horizontal bars*, timing of capecitabine administration; *arrows*, timing of docetaxel injection. ◇, vehicle; △, capecitabine alone; □, docetaxel alone; ■, capecitabine and docetaxel in combination.

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 ($3 \times 10^5$ viable cells/mouse) was inoculated s.c. into female C57BL/6 mice. A suspension of WiDr tumor cells ($5 \times 10^6$ 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, $V = ab^2/2$, 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

provided by Rhone-Poulenc Rorer (Antony, France). The other cytostatic drugs were purchased from the following suppliers: UFT from Taiho Pharmaceutical Co. (Tokyo, Japan); 5-FUra and Adriamycin from Kyowa Hakko Co. (Tokyo, Japan); cyclophosphamide from Shionogi (Osaka, Japan). Capecitabine was dissolved in 40 mM citrate buffer (pH 6.0) containing 5% gum arabic as the vehicle and given p.o. 5′-dFUrd and UFT were dissolved or suspended in 5% gum arabic and given p.o. Docetaxel was dissolved in saline containing 2.5% ethanol and 2.5% polysorbate 80 and given i.v. 5-FUra, Adriamycin, and cyclophosphamide were dissolved in saline and given i.p. MTDs of fluoropyrimidines indicated in Table 1 were based on the data obtained from nude mice bearing human colon tumor HCT 116 in previous studies (8, 15). The highest nonlethal dose of docetaxel single injection in mouse was 285 mg/m$^2$, which corresponds to 114 mg/kg, whereas the MTD of four consecutive injections in a week was 13.4 mg/kg/day (16).

**dThdPase Level in Tumor Tissues and PyNPase Activity Assays.** With a glass homogenizer, tumor tissues were homogenized in 10 mM Tris buffer (pH 7.4) containing 15 mM NaCl, 1.5 mM $MgCl_2$, and 50 μM potassium phosphate. The homogenate was then centrifuged at 10,000 × *g* for 20 min at 4°C, and the supernatants were stored at −80°C until used. The protein concentration of the supernatant was determined using a DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA). The dThdPase level was measured by ELISA with monoclonal antibodies specific to human dThdPase as described previously (17). This ELISA system does not cross-react with mouse dThdPase. One unit corresponds to the dThdPase level of the standard enzyme solution (extracts of human colon cancer xenograft HCT116), which catalyzes phosphorolysis of 5′-dFUrd and generates 5-FUra at a rate of 1 μg of 5-FUra per h. For the measurement of PyNPase activities in murine tumor and normal tissues, the homogenate was centrifuged at 105,000 × *g* for 90 min at 4°C, and the supernatants were then dialyzed overnight against 20 mM potassium phosphate buffer (pH 7.4) containing 1 mM β-mercaptoethanol. PyNPase activity converting 5′-dFUrd into 5-FUra, carried by dThdPase and uridine phosphorylase, was measured as reported before (18, 19).

## 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.

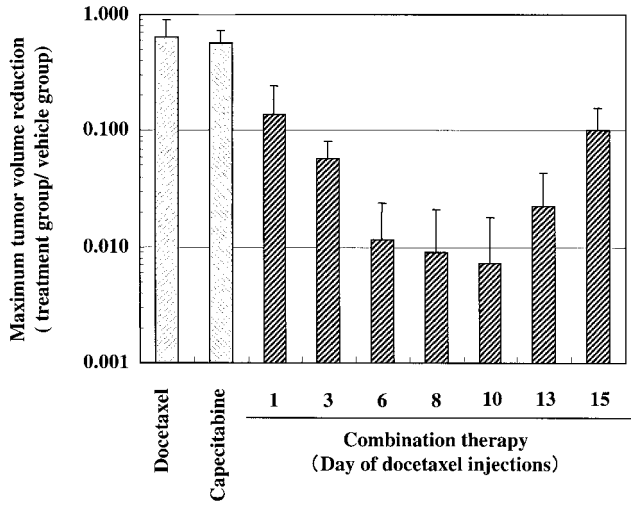


*Fig. 2* Maximum inhibitory rate of tumor growth induced by the combination of docetaxel and capecitabine in different regimens in MX-1 human mammary tumor xenografts. Mice bearing MX-1 tumors were randomized into groups of five mice each. Capecitabine was administered p.o. for 14 days (days 1–14) at 359 mg/kg beginning 15 days after the tumor inoculation, whereas docetaxel (7.5 mg/kg) was injected i.v. once on day 1, day 3, day 6, day 8, day 10, day 13, or day 15. As a control group of docetaxel alone, docetaxel was injected on day 8. Tumor volume was measured three times a week. The volume of the treated groups was compared with that of the vehicle group on the same day. Maximum reduction of the group was plotted [mean ± SD (*bars*)].

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-

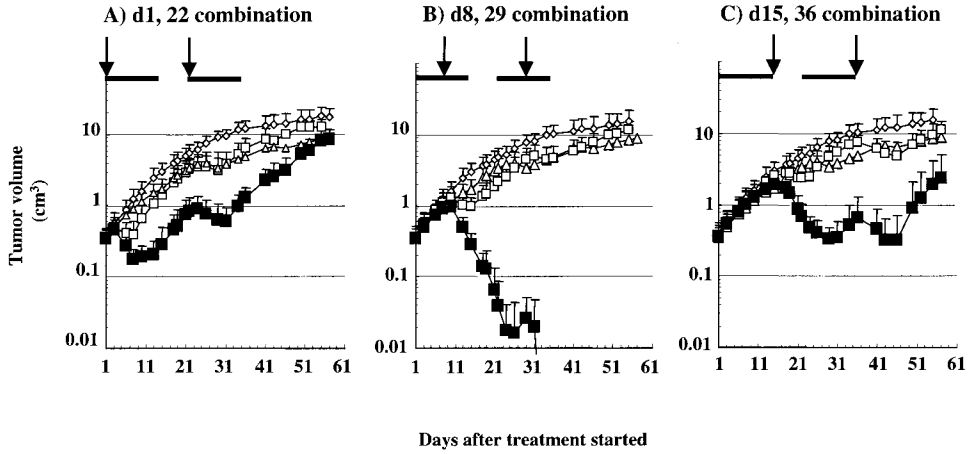


*Fig. 3* Antitumor activity of repeated combination regimens with capecitabine and docetaxel on tumor growth of MX-1 human mammary tumor xenografts. Mice bearing MX-1 tumors were randomized into groups of six mice each. Three weeks of combination regimens described in Fig. 1 were repeated twice beginning 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*) capecitabine 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.

nation (Fig. 2). Docetaxel injected on day 1, day 3, day 13, or day 15 was less potent than the optimal combination regimens with a single docetaxel injection during day 6 through day 10.

Superiority of the day 8 combination regimen over the day 1 or day 15 combination regimens was reproduced in repeated-treatment regimens as well (Fig. 3). All tumors in the day 8 combination group were completely regressed after two cycles of the 3-week treatment. On the other hand, the second cycle of the day 1 combination or the day 15 combination showed similar activity to that of their first cycle. Although a temporary reduction in tumor volume was observed after docetaxel injection in every regimen, the reduction was more pronounced when docetaxel was injected in the middle of the capecitabine treatment (day 8 combination regimen).

**Optimal Dosing Regimen for Combination Therapy with Docetaxel and Other Agents.** We next examined the schedule dependency of docetaxel in combination with other agents. Fig. 4 shows the results of 5′-dFUrd and 5-FUra. Doses of 5′-dFUrd and 5-FUra used in these experiments, 123 and 13 mg/kg, are two-thirds of their MTD, respectively (15), whereas that of docetaxel, 7.5 mg/kg, was 1/15 of the highest nonlethal dose (16). Similar to capecitabine, the day 8 combination of docetaxel with 5′-dFUrd showed much stronger antitumor activity than the day 1 or day 15 combination. In the day 8 combination group, complete regression of the tumor was found in three of six mice at 2 weeks after termination of the 5′-dFUrd treatment. In the case of a combination with docetaxel and 5-FUra, the day 8 combination also exhibited better efficacy compared with the day 1 or day 15 combination. However, the extent of the tumor volume reduction was weaker than that found in capecitabine or 5′-dFUrd plus docetaxel.

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 $mm^3$ 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-

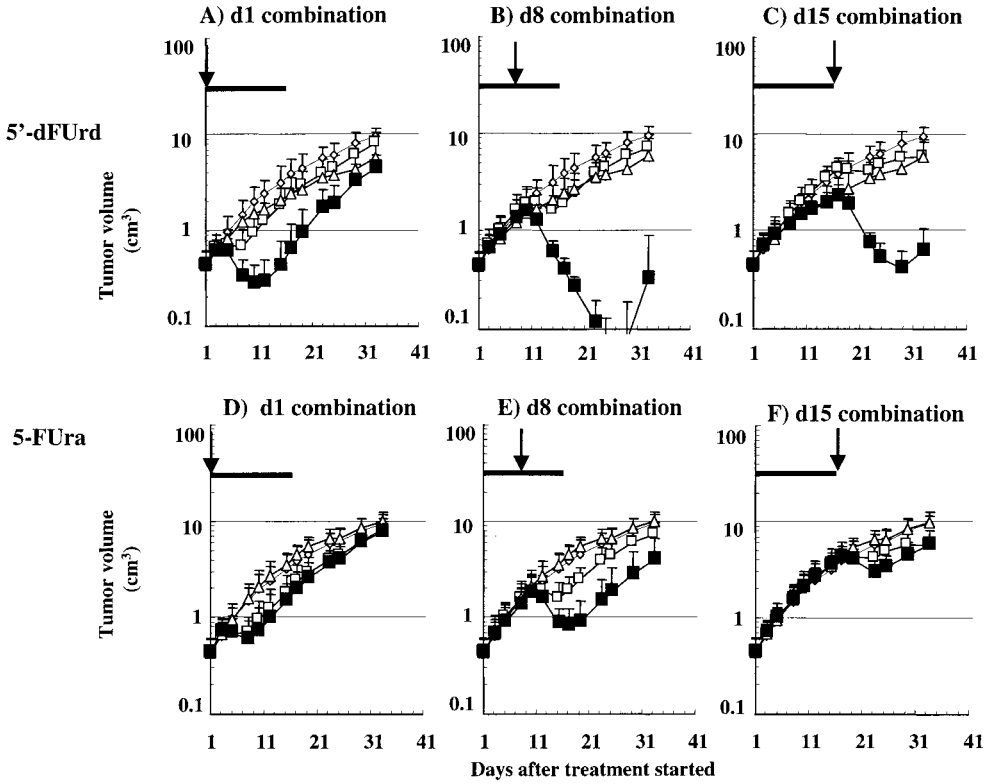


*Fig. 4* Comparison of antitumor activity in different regimens in combination with 5′-dFUrd or 5-FUra and docetaxel in MX-1 human mammary tumor xenografts. Mice bearing MX-1 tumors were randomized into groups of 6 mice each. 5′-dFUrd (123 mg/kg, *A–C*) or 5-FUra (13 mg/kg, *D–F*) were administered p.o. for 14 days beginning 18 days after the tumor inoculation, whereas docetaxel (7.5 mg/kg) was injected i.v. either on the first day (*A* and *D*, *d1 combination*), on the eighth day (*B* and *E*, *d8 combination*) or on the next day (*C* and *F*, *d15 combination*) of 5′-dFUrd or 5-FUra administration. Data are mean ± SD (*vertical bars*) of tumor. *Horizontal bars*, timing of 5′-dFUrd or 5-FUra administration; *arrows*, timing of docetaxel injection. ◇, vehicle; △, 5′-dFUrd or 5-FUra alone; □, docetaxel alone; ■, 5′-dFUrd or 5-FUra and docetaxel in 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 addition, in the A755 model, the growth delay in the day 5 combination was more potent than other regimens. Thus, administration of docetaxel in the middle of capecitabine or 5′-dFUrd treatment seems to be an effective regimen in three mammary tumor models. In contrast, in the human colon tumor WiDr model, the day 1 combination was better than the day 8 combination.

**Toxicity in Different Dosing Regimens.** In the experiments with docetaxel and capecitabine/5′-dFUrd in the MX-1 model described above, we have not observed severe toxicity in any of three different combination regimens. However, in these experiments we have used low doses of docetaxel to clearly demonstrate schedule-dependent antitumor activity. To clarify the effect on toxicity of different dosing regimens, we compared three regimens at much higher doses (one-fourth of highest nonlethal dose) of docetaxel in combination with MTD of 5′-dFUrd, in MX-1-bearing mice. As shown in Table 2, the day 8 combination did not induce stronger toxicity, in terms of carcass weight loss and reduction in peripheral blood leukocyte count, compared with the day 1 or day 15 combinations. In addition, none of the groups showed gastrointestinal toxicity assessed by occult blood test and fecal observation (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,

*Table 1* Tumor growth delayed by the combination of docetaxel and fluoropyrimidines in MX-1 human mammary tumor xenograft

Mice bearing MX-1 were randomized into groups of five or six mice each, and treatment was started 14 days after tumor inoculation. Capecitabine, 5′-dFUrd, or UFT was administered p.o., and 5-FUra was administered i.p. daily for 14 days, whereas docetaxel (7.5 mg/kg) was injected i.v. on the eighth day of fluoropyrimidine treatment. $T_{2000}$ is the time (days) taken for a tumor to grow to 2000 $mm^3$ from the day of tumor inoculation.

| | | Fluoropyrimidine alone | | Combination with docetaxel | | |
|---|---|---|---|---|---|---|
| Drugs | Doses (mg/kg) | $T_{2000}$ | Growth delay[a] (days) | $T_{2000}$ | Growth delay[a] (days) | Complete regression[b] (mice/group) |
| Vehicle | | 24.5 ± 2.7[c] | | 32.7 ± 3.9 | 8.2 | 0/6 |
| Capecitabine | 359 (2/3 MTD) | 28.3 ± 3.1 | 3.8 | 64.2 ± 8.0 | 39.7 | 3/6 |
| | 539 (MTD) | 30.8 ± 3.9 | 6.3 | >84 | >59.5 | 5/6 |
| 5′-dFUrd | 123 (2/3 MTD) | 32.9 ± 5.3 | 8.4 | >84 | >59.5 | 3/5 |
| | 185 (MTD) | 34.8 ± 6.5 | 10.3 | >84 | >59.5 | 5/6 |
| 5-FUra | 13 (2/3 MTD) | 26.8 ± 3.0 | 2.3 | 47.8 ± 13.6 | 23.3 | 1/6 |
| | 20 (MTD) | 29.3 ± 5.5 | 4.8 | 48.2 ± 5.0 | 23.7 | 0/6 |
| UFT | 13 (2/3 MTD) | 26.5 ± 1.8 | 2.0 | 44.8 ± 12.2 | 20.3 | 0/6 |
| | 20 (MTD) | 27.7 ± 4.2 | 3.2 | 53.3 ± 11.7 | 28.8 | 1/6 |
| | 30 (3/2 MTD) | 32.0 ± 5.3 | 7.5 | Toxic | | Toxic |

[a] Difference in $T_{2000}$ between each group and vehicle group.
[b] Number of mice per group, the tumors of which were completely regressed on day 84, 8 weeks after the final administration of fluoropyrimidines. None of the mice in fluoropyrimidine-alone groups reached complete regression.
[c] Mean ± SD.

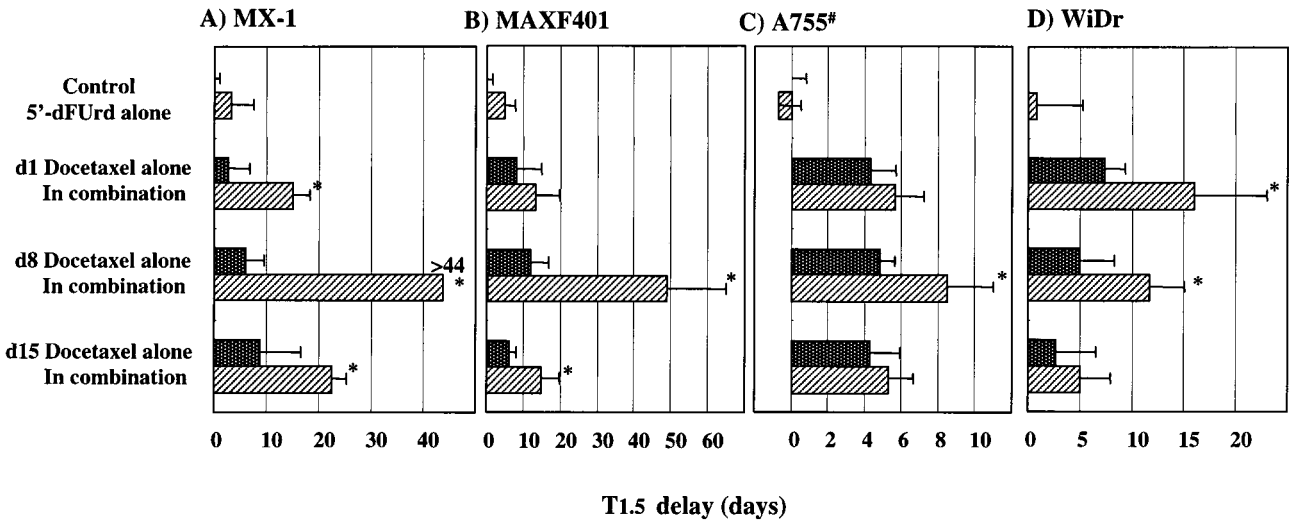


*Fig. 5* Tumor growth delay induced by the combination of docetaxel and 5′-dFUrd in different regimens in four tumor models. Delay in time (days) taken for a tumor to grow to 1.5-fold of volume from the day of docetaxel injection or from day 15 in the case of 5′-dFUrd alone is indicated [$T_{1.5}$ delay, mean values ± SD (*bars*)]. *A*, human mammary tumor MX-1 model. 5′-dFUrd (123 mg/kg p.o. on day 1 through day 14) and docetaxel (7.5 mg/kg i.v. on day 1, day 8, or day 15) were administered to the mice bearing MX-1 (6 mice/group), beginning 18 days after the tumor inoculation. *B*, human mammary tumor MAXF401 model. 5′-dFUrd (123 mg/kg p.o. day 1through day 14) and docetaxel [15 mg/kg i.v. on day 1 (*d1*), day 8 (*d8*), or day 15 (*d15*)] were administered to the mice bearing MAXF401 (six mice/group), beginning 21 days after the tumor inoculation. *C*, murine mammary tumor A755 model. 5′-dFUrd (246 mg/kg p.o. on day 1 through day 8) and docetaxel (10 mg/kg i.v. on day 1, day 5, or day 9) were administered to the mice bearing A755 (six mice/group), beginning 12 days after the tumor inoculation. *D*, human colon tumor WiDr model. 5′-dFUrd (246 mg/kg, p.o. day 1 through day 14) and docetaxel (15 mg/kg i.v. on day 1, day 8, or day 15) were administered to the mice bearing WiDr (six mice/group) beginning 11 days after the tumor inoculation. *, significantly different from the corresponding docetaxel alone group; $P < 0.05$ by Mann-Whitney *U* test. #, In the case of A755, docetaxel was injected on day 1, day 5, or day 9, and 5′-dFUrd was administered daily from day 1 to day 8.

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 2* Toxicity of three different regimens in combination with 5′-dFUrd 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′-dFUrd (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.

| | Peripheral blood leukocyte count ($\times 10^6$ cells/ml of blood) | | | Carcass wt (g) | | |
|---|---|---|---|---|---|---|
| Administration | Day 8 | Day 15 | Day 22 | Day 8 | Day 15 | Day 22 |
| Vehicle | 7.45 ± 1.33 | 8.80 ± 1.09 | 11.40 ± 1.59 | 18.8 ± 1.3 | 20.3 ± 1.5 | 18.1 ± 0.7 |
| 5′-dFUrd alone | 3.63 ± 0.50 (49)[a] | 5.55 ± 0.21 (63) | 8.30 ± 1.34 (73) | 17.6 ± 1.4 | 19.0 ± 2.6 | 21.7 ± 0.7 |
| Docetaxel alone | 8.75 ± 3.29 (117) | ND[b] | ND | 17.9 ± 1.1 | ND | ND |
| Day 1 combination | 2.13 ± 0.57 (29) | 4.70 ± 2.82 (53) | 9.83 ± 1.79T(86) | 17.1 ± 0.8 | 19.1 ± 1.4 | 21.6 ± 1.2 |
| Day 8 combination | | 5.73 ± 2.37 (65) | 11.53 ± 1.94 (101) | | 16.9 ± 1.8 | 22.2 ± 2.1 |
| Day 15 combination | | | 10.33 ± 3.40 (154) | | | 19.3 ± 1.9 |

[a] Numbers in parentheses, percentage of treated *versus* vehicle.
[b] 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 7 days later, and concentrations of dThdPase were determined by ELISA. C57BL/6 mice bearing A755 mammary carcinoma were given i.v. injection of docetaxel, and 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.

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

[a] 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′-dFUrd 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′-dFUrd and paclitaxel in combination. Their efficacy was also more than merely additive when paclitaxel was given in the day 8 regimen in the MX-1 model (data not shown).

The effect of combinations observed in the present study *in vivo* is distinct from that reported elsewhere in *in vitro* studies (2–4), where the efficacy of paclitaxel and 5-FUra was less than additive or antagonistic. One possible explanation for this discrepancy is the use of different tumor cell lines. However, we have observed no antagonistic activity of taxanes and capecitabine in any tumor models studied *in vivo*. Another explanation is that the synergy observed in the day 8 regimen would result from *in vivo* specific actions of either capecitabine/5′-DFUR or docetaxel. We have not yet tested the combination *in vitro* because the MX-1, MAXF401, and A755 lines, in which a potent *in vivo* effect was observed, cannot grow *in vitro*. It is, however, likely that either capecitabine/5′-dFUrd or docetaxel enhances the efficacy of either drug *in vivo*.

One possible mechanism of the synergy *in vivo* is that docetaxel up-regulates dThdPase, the enzyme essential for the activation of 5′-dFUrd to 5-FUra, and consequently enhances the efficacy of 5′-dFUrd 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′-dFUrd, 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′-dFUrd 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/m$^2$ in humans, 5′-dFUrd at 123 mg/kg corresponds to 569 mg/m$^2$ in humans, and docetaxel at 7.5–15 mg/kg corresponds to 35–70 mg/m$^2$ 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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# Schedule Dependency of Antitumor Activity in Combination Therapy with Capecitabine/5′-Deoxy-5-fluorouridine and Docetaxel in Breast Cancer Models

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