

Combination of Oral Fluoropyrimidine and Docetaxel: Reappraisal of Synergistic Effect Against Gastric Carcinoma Xenografts

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Abstract. *Background:* The synergistic antitumor effect of a combination of docetaxel and capecitabine is reported to be attributable to docetaxel-mediated up-regulation of thymidine phosphorylase (dThdPase). *Materials and Methods:* Intravenous docetaxel (15 mg/kg) was given to nude mice bearing xenografts of the gastric cancer cell lines MKN45 and MKN28. Mice were sacrificed on days 7, 10 and 22 and tumor samples were taken to measure the activities of thymidylate synthase, dihydropyrimidine dehydrogenase, dThdPase and orotate phosphoribosyltransferase. The efficacy of capecitabine or S-1, alone and in combination with docetaxel, was then evaluated *in vivo*. Docetaxel was administered intravenously on days 8 and 22 at 15 mg/kg, while capecitabine (269 mg/kg) or S-1 (7.5 mg/kg) were administered orally 5 times a week for 4 weeks. *Results:* Tumor regression was observed only for a combination of capecitabine and docetaxel against MKN28, while additive growth inhibition was obtained by the combination of docetaxel and both S-1 and capecitabine on MKN45 tumor xenografts. Induction of dThdPase activity was observed only for MKN45. The activity of no other enzyme was significantly affected following administration of docetaxel. *Conclusion:* The combination of oral fluoropyrimidine and docetaxel showed augmented antitumor activity, but this may be attributed to mechanisms other than changes in 5-fluorouracil-metabolizing enzymes.

Gastric carcinoma remains incurable when diagnosed as an unresectable disease, and the median survival of patients

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treated with chemotherapy in numerous phase II trials seldom exceeds a year (1). Although intravenous 5-fluorouracil (5-FU) had been a key drug for the treatment of cancer of the gastrointestinal tract for several decades, oral administration of prodrugs and other 5-FU-related drugs were recently found to be superior, or at least equivalent, to the intravenous administration in terms of survival benefit for colorectal cancer (2, 3). S-1, a mixture of tegafur (FT), 5-chloro-2,4-dihydropyridine (CDHP) and potassium oxonate (Oxo), achieved a remarkable response rate of over 40% in phase II trials (4, 5), and is widely used in Japan for the treatment of advanced gastric cancer, alone and in combination with other drugs (6). Capecitabine generates 5-FU selectively in tumors by three enzymes located in the liver and in the tumors (7). The final step is the conversion of the intermediate metabolite 5'-deoxy-5-fluorouridine to 5-FU by thymidine phosphorylase (dThdPase) in tumors. Taxanes, another new category of drugs with activity against cancer (8), are known to up-regulate the dThdPase levels in cancer tissues, thus significantly enhancing the activity of capecitabine when used in combination (9). This synergistic effect has been recognized *in vivo* using breast and colorectal cancer cell lines, and an optimal treatment schedule for an *in vivo* model has been elucidated (9, 10). This combination has also been tested in several phase II trials and was found to be promising (11, 12). In the current study, a gastric cancer xenograft was used for the first time to obtain a rationale for the combination of capecitabine and docetaxel for treatment of this cancer type, and also to compare the effect of capecitabine with S-1, the drug most frequently used for treatment of advanced gastric cancer in Japan, at doses commonly used *in vivo*.

Materials and Methods

Animals. Seven- to eight-week-old male athymic nude mice of the KSN strain were purchased from from Shizuoka Laboratory

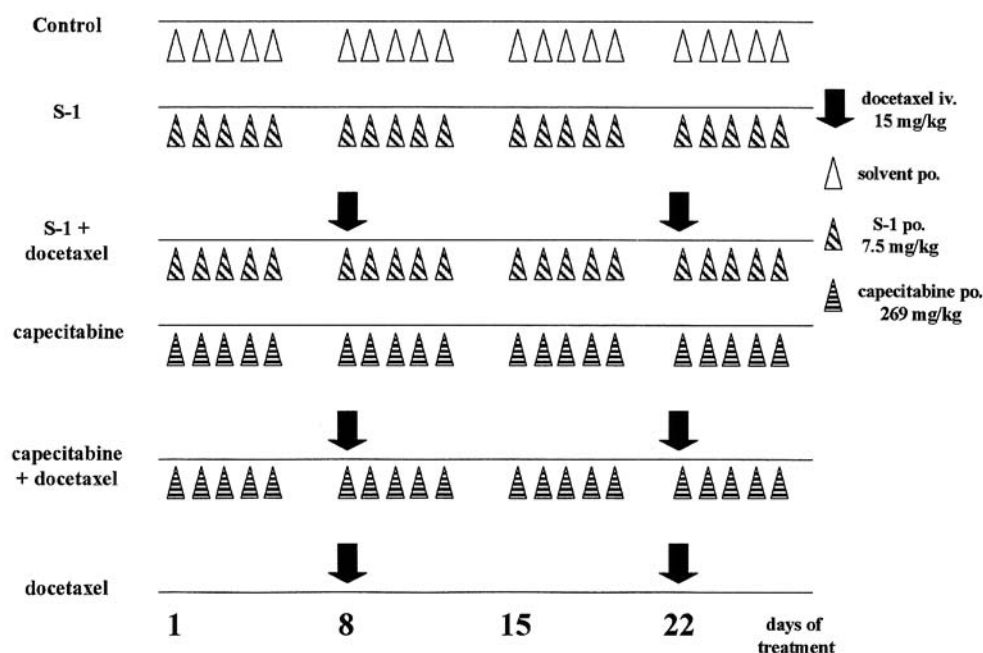


Figure 1. Schematic representation of 6 experimental protocols. Five mice each were treated with oral S-1 (containing 7.5 mg/kg/day tegafur) or oral capecitabine at a dose of 269 mg/kg/day 5 days a week for 4 weeks, either alone or in combination with biweekly intravenous docetaxel at a dose of 15 mg/kg. Another 5 mice were treated with biweekly intravenous docetaxel at a dose of 15 mg/kg. Five control mice were treated with oral administration of solvent.

Animal Center (Hamamatsu, Japan) and maintained under specific pathogen-free conditions.

Cell lines. The human gastric cancer cell lines MKN28 and MKN45 were purchased from the Riken Cell Bank (Tsukuba, Japan). They were maintained in cultures with Rosewell Park Memorial Institute (RPMI)-1640 medium containing 10% fetal calf serum, at 37°C under 5% CO₂. IC₅₀ values of 5-FU for MKN28 and MKN45, as evaluated by the modified tetrazolium salt (MTT) assay, were 2.5 µM and 0.14 µg/mL, respectively, while those of docetaxel were below 0.002 µg/mL for each of the cell lines.

Chemicals. Capecitabine was purchased from Roche Pharmaceutical Company through Chugai-Pharm Co. Ltd. (Tokyo, Japan). Docetaxel was purchased from Aventis Pharma Japan (Tokyo, Japan) and dissolved in saline containing 2.5% ethanol and 2.5% polysorbate 80. Tegafur (FT), 5-chloro-2,4-dihydropyridine (CDHP) and potassium oxonate (Oxo) were supplied by Taiho Pharmaceutical Co. Ltd. (Tokyo, Japan). S-1 was prepared by mixing FT, CDHP and Oxo in a molar ratio of 1:0.4:1 and suspending them in 0.5% sodium hydroxypropylmethylcellulose (HPMC). [6-³H]-5-FU (525 Gbq/mmol), [6-³H]-thymidine (dThd; 2.41 Tbq/mmol) and [6-³H]-FdUMP (625 Gbq/mmol) were obtained from Moravex Biochemicals, Inc. (CA, USA).

Human cancer xenograft model and treatment schedule. Cells in the logarithmic growth phase were detached, and 1x10⁶ cells were subcutaneously inoculated with a trocar needle into the dorsal flank of each mouse. To evaluate the antitumor effect of the

anticancer agents, the tumor size and body weight of the mice were measured 3 times a week. The tumor volume was estimated by using the following equation, $V=ab^2/2$, where a and b are tumor length and width, respectively. The experiments for each cell line were started when the tumor volume reached approximately 0.5 and 0.7 cm³, respectively. For the evaluation of cytostatic efficacy, tumor volume change was expressed by the formula VT/V₀, where VT is the mean volume on any given day and V₀ is the mean volume at the start of administration. The carcass body weight was calculated by subtracting the tumor weight, which was estimated from the tumor volume, from the body weight. All animal experiments were carried out in accordance with the Guidelines for the Welfare of Animals in Experimental Neoplasia.

The treatment schedules for monotherapies and combined chemotherapies are shown in Figure 1. Mice underwent oral administration through a gastric tube of either S-1 containing 7.5 mg/kg/day of tegafur or capecitabine at a dose of 269 mg/kg/day, for 5 days a week. The treatment was continued for 4 weeks. Docetaxel was given intravenously at a dose of 15 mg/kg on days 8 and 22, alone and in combination with the oral agents. This schedule was based on a report that administration of docetaxel on day 8 had resulted in the greatest efficacy with oral capecitabine or 5-FU during 2 weeks of treatment (10). Control mice were treated with the solvent alone. Each treatment was performed for 5 mice.

Measurements of enzymatic activities of xenografts pretreated with docetaxel. To evaluate changes in the activities of various enzymes that are associated with the metabolism of fluorouracil and related compounds following administration of docetaxel, 15 mice for each

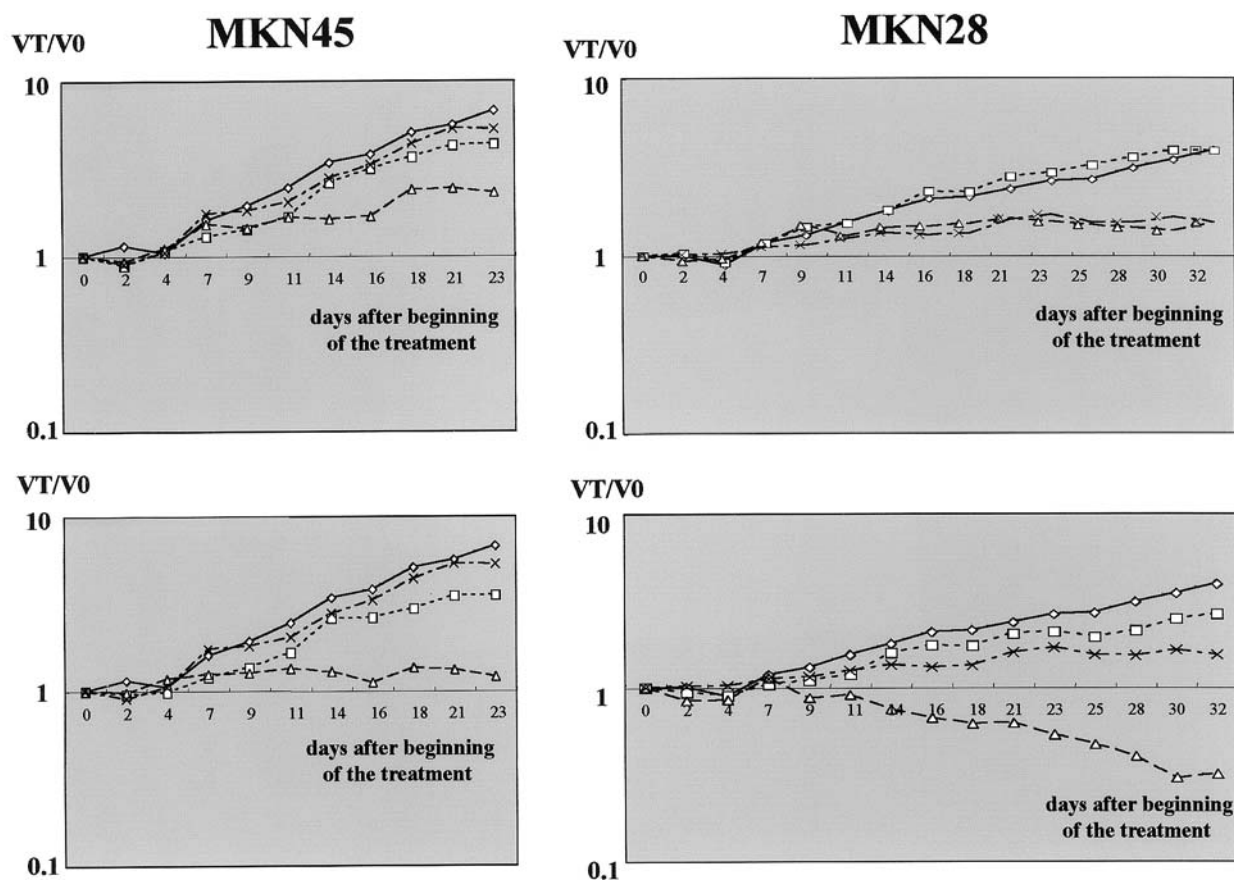


Figure 2. Antitumor activity of the 6 protocols on xenografts of gastric cancer cell lines MKN45 and MKN28. Mice were treated with S-1 (above) or capecitabine (below) alone and in combination with intravenous docetaxel; \diamond - \diamond control, \times - \times docetaxel alone, \square - \square oral fluoropyrimidine alone, \triangle - \triangle oral fluoropyrimidine in combination with docetaxel. Five mice were treated by each of the 6 protocols.

xenograft underwent treatment with intravenous administration of 15 mg/kg docetaxel. Five mice each were sacrificed 7, 10 and 22 days after the treatment with docetaxel, and the tumors were harvested and snap-frozen for measurement of various enzymatic activities. Xenografts from 5 untreated mice were harvested as control samples.

Snap-frozen tumors were thawed and homogenized with 3 volumes of 50 mM Tris-HCl (pH 7.6) containing 10 mM 2-mercaptoethanol, 25 mM KCl and 5 mM MgCl₂, centrifuged at 105,000 x g for 60 min, and the resulting supernatant was used to measure enzyme activity. TS was measured by [6-³H]-FdUMP binding assay based on the method of Spears *et al.* (13). OPRT activity was determined according to the method of Shirasaka *et al.* (14), using [6-³H]-5-FU as the substrate. TP was measured according to the modified method described by Maehara *et al.* (15). Briefly, the reaction mixture, containing 50 mM potassium phosphate buffer (pH 7.0), 0.6 mM [6-³H]-dTd (74 Kbp) and 50 μ L of enzyme extract in a final volume of 0.125 mL, was incubated at 37°C for 30 min, and immediately heated at 100°C for 2 min, followed by centrifugation. Then, 10- μ L aliquots of the supernatant were subjected to thin layer chromatography. DPD activity was determined using [6-³H]-5-FU as the substrate, according to the method described previously (15).

Results

The antitumor effects against each xenograft of the oral agents alone and in combination with intravenous docetaxel are shown in Figures 2. The changes in carcass body weight of mice treated with each experimental protocol were trivial for all treatment schedules. MKN45 grew rapidly in the absence of antineoplastic agents and was relatively resistant to docetaxel. In contrast, both oral fluoropyrimidines showed antitumor activities, which were further enhanced by combining with docetaxel. Treatment by a combination of capecitabine and docetaxel resulted in a stable disease. The MKN28 xenograft was slow-growing compared with MKN45, and was resistant to oral fluoropyrimidines, and S-1 in particular, whereas docetaxel was somewhat more active. The combination chemotherapy with S-1 and docetaxel showed no synergic effect and the activity was similar to the treatment with docetaxel alone. By far the greatest response was observed by the combination of

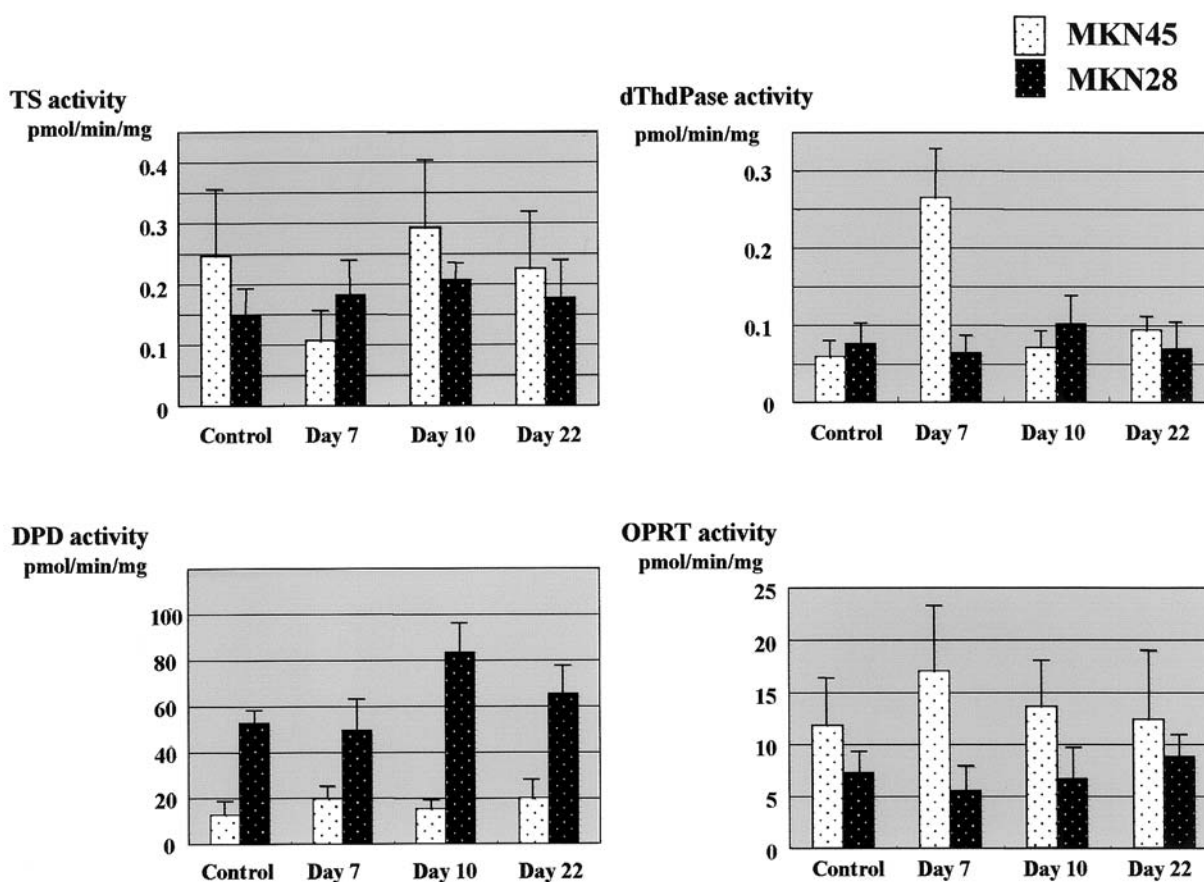


Figure 3. Profiles of enzymatic activities of the gastric cancer xenografts MKN45 and MKN28 pretreated with intravenous docetaxel at a dose of 15 mg/kg.

capecitabine and docetaxel, which resulted in regression of the xenograft.

Pretreatment with docetaxel resulted in induction of dThdPase activity by almost 5-fold on the seventh day for MKN45 (Figure 3), but this effect was transient. A slight increase in the enzymatic activity of OPRT and decrease in TS were also observed on day 7, while DPD remained relatively stable throughout the observation period (Figure 3). MKN28 was characterized by inherently higher enzymatic activity of DPD, and this was further enhanced on day 10. The activities of the other enzymes remained relatively stable.

Discussion

Combining several anticancer drugs is a common practice for treatment of solid cancers. Combination therapies using fluoropyrimidines and taxanes are among several combinations with activity against solid cancers and a rationale for this combination seems to have been

established; pretreatment with the taxanes up-regulates intratumoral dThdPase, which converts capecitabine and 5'-deoxy-5-fluorouridine into 5-FU (9). However, such a synergistic effect *in vivo* had not been reported using gastric cancer xenografts. In addition, S-1, a novel oral fluoropyrimidine, reportedly inhibits DPD, an enzyme that metabolizes 5-FU, and has shown a high response rate for advanced gastric cancer with measurable lesions (4, 5). This drug has also been used in combination with the taxanes (16, 17), but the rationale for this combination had not been elucidated. The results of the current study, however, failed to solve several queries and seem to point to the fact that the mechanisms of drug sensitivity and resistance are complex and multifactorial, and that combination therapies do not always produce synergistic effects.

For both of the cell lines, the combination of intravenous docetaxel and oral fluoropyrimidines was more active than monotherapies with either of the drugs. As for MKN45, little therapeutic effect was observed from monotherapies with S-1, capecitabine, or docetaxel. The

addition of docetaxel to either of the fluoropyrimidines resulted in a marked enhancement of the activity, particularly for capecitabine, which resulted in prolonged growth suppression of the xenograft. The enhanced activity of capecitabine may be attributable to the induction of dThdPase activity by 5-fold. In addition, mild up-regulation of OPRT and slight down-regulation of TS may have had some influence on the activity of the two combination therapies.

On the other hand, a greater difference in sensitivity was observed against the two oral fluoropyrimidines for MKN28. Capecitabine was markedly more active than S-1, both as a single agent and in combination with docetaxel. This was surprising given that MKN28 was a xenograft with relatively high DPD activity and S-1, which contains a DPD inhibitor CDHP, had been designed to overcome 5-FU resistance due to the DPD. Inherent DPD activity in MKN28 was 4-fold higher than in MKN45, and this activity was further enhanced by nearly 2-fold on day 10 from the administration of docetaxel. No apparent up-regulations or down-regulations were observed for other fluoropyrimidine-related enzymatic activities. These enzymatic profiles were no drawback to the effect of combination therapy with docetaxel and capecitabine, which induced a constant tumor regression.

The most enigmatic result regarding MKN28 was that S-1 was no more active than the control group treated with the solvent. In addition, S-1 in combination with docetaxel was no more active than a treatment with docetaxel alone. There is currently no way of explaining this phenomenon solely from the viewpoint of enzymatic activities. The dose of S-1 at 7.5 mg/kg/day could have been suboptimal in contrast to the dose of capecitabine. However, Nishimura *et al.* showed that a dose of 7.5 mg/kg is optimal for use in *in vivo* treatment that continues for 4 weeks (18). Fujiwara *et al.* used a dose of 10 mg/kg for a treatment that continued for 9 days (19). The dose of 7.5 mg/kg given 5 days a week for 4 weeks would then seem reasonable, especially because it was combined with docetaxel at a dose 2-fold higher than the dose used by Fujimoto-Ouchi *et al.* in the similar combination chemotherapy (10). In their *in vivo* study, Fujiwara *et al.* used cell lines in which the DPD activity was more than 10-fold that of MKN28. It was for these cell lines that S-1 really exhibited an advantage over 5-FU. This suggests that the relative 5-FU resistance of MKN28 in comparison with MKN45 may be attributable to factors other than the 4-fold increase in the activity of DPD for MKN28.

To conclude, the combination therapies with oral fluoropyrimidines and taxanes were found to have at least an additive effect against gastric cancer xenografts and were more active than monotherapies with any of the three drugs tested. Up-regulation of dThdPase activity may, in part, have been responsible for the enhanced efficacy of the

docetaxel/capecitabine combination against MKN45. However, the synergistic effect, or the lack of it, could not be explained solely on the basis of regulation of 5-FU-related enzymes in the case of MKN28, and this may be the case in several gastric cancer patients in the clinical setting.

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