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In vitro schedule-dependent interaction between paclitaxel and SN-38 (the active metabolite of irinotecan) in human carcinoma cell lines

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Abstract Paclitaxel and irinotecan are important new anticancer agents. The combination of these two agents has been considered for use against a variety of advanced solid tumors. Since the schedule-dependent effects of this combination may be crucial to its use, we studied the interaction of paclitaxel and SN-38 (the active metabolite of irinotecan) in various schedules in four human cancer cell lines in culture. Cell growth inhibition after 5 days was determined using an MTT assay. The effects of drug combinations at the IC_{80} level were analyzed by the isobologram method. Simultaneous exposure to paclitaxel and SN-38 for 24 h produced antagonistic (subadditive and protective) effects in the human lung cancer cell line A549, the breast cancer cell line MCF7, and the colon cancer cell line WiDr, and produced additive effects in the ovarian cancer cell line PA1. Sequential exposure to paclitaxel for 24 h followed by SN-38 for 24 h, and the reverse sequence, produced additive effects in all four cell lines. These findings suggest that sequential administration, not simultaneous administration, may be the appropriate schedule for the therapeutic combination of paclitaxel and irinotecan. Continued preclinical and clinical studies should provide further insights and assist in determining the optimal schedule for this combination in clinical use.

Key words Paclitaxel · Irinotecan · SN-38 · Isobologram

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Introduction

Combination chemotherapy has improved the response and survival rates of patients with hematological malignancies and some solid tumors; however, for many solid tumors, such improvements have been marginal. This may be because of the lack of active single agents. The identification of new agents is critical for further progress in the treatment of such tumors, and the evaluation of many new compounds is underway. Combining these agents effectively may lead to significant therapeutic advances.

Paclitaxel and irinotecan are promising candidates for such anticancer agents. Paclitaxel is an antimicrotubular agent isolated from the bark of the Pacific yew tree, *Taxus brevifolia*. This agent exerts cytotoxic effects by promoting and stabilizing microtubule assembly, thus interrupting the dynamic cellular reorganization necessary for mitosis [34]. The primary dose-limiting toxicity of paclitaxel has been found to be myelosuppression, mainly neutropenia; other toxicities include hypersensitivity reactions, neuropathy, mucositis, mild nausea and vomiting, and arrhythmias [5]. Paclitaxel has been confirmed to have significant activity against lung, breast, and ovarian cancers [2, 8, 25, 27], and clinical studies of its effects on several other cancers are in progress.

Irinotecan is an analogue of camptothecin [18], a plant alkaloid isolated from the Chinese tree, *Camptotheca accuminata*. Although clinical trials of camptothecin were performed more than 20 years ago, minimal antitumor activity and severe toxicities halted further study [6, 26]. Camptothecin has since been found to have a novel mechanism of action targeting topoisomerase-I, which relieves torsional strain in DNA by inducing reversible single-strand breaks [9]. This finding has led to the development of the soluble semisynthetic analogues of camptothecin, irinotecan and topotecan. Clinical studies of irinotecan have shown that the dose-limiting toxicities are myelosuppression, mainly neutropenia, and diarrhea [38]. Irinotecan has shown significant

activity against lymphoma, and lung, gastrointestinal, and gynecological cancers with tolerable toxicity [23, 24, 29, 35, 39].

The rationale for the clinical trials of paclitaxel and camptothecin derivatives in combination is based on their high activity against a variety of cancers, different cytotoxic mechanisms, absence of cross-resistance [11], and nonoverlapping toxicites (except for neutropenia). Phase I studies of paclitaxel and topotecan in combination are now in progress [20, 30]. A clinical study of paclitaxel and irinotecan is also being planned. However, no preclinical data are available delineating differences in antitumor activity and toxicity related to the sequence of administration of this combination. Paclitaxel and camptothecin are cell cycle-specific agents. Paclitaxel blocks the cells in the G_2/M phase [22, 31], whereas the sensitivity of cells to camptothecin is highest during their progression through the S phase, and the drug delays the cell cycle traverse in the S/G_2 phase [4, 28]. Disturbances of the cell cycle by combinations of these two agents may show antagonistic or synergistic effects and the drug schedule may be an important determinant of the activity. To obtain preclinical data regarding the effects of this combination, we studied the antitumor interaction between paclitaxel and 7-ethyl-10-hydroxycamptothecin (SN-38) [17], the active metabolite of irinotecan, in various schedules in four human solid tumor cell lines in vitro.

The cells were exposed to the drugs for 24 h, since paclitaxel and irinotecan are often clinically administered by infusion over a few hours or by continuous infusion, and the cytotoxic plasma levels of these agents are maintained for more than 10 h.

Materials and methods

Cell lines

Experiments were conducted with four human carcinoma cell lines: the non-small-cell lung cancer cells, A549, the breast cancer cells, MCF7, the ovarian cancer cells, PA1, and the colon cancer cells, WiDr. These cells were maintained in 75-cm^3 plastic tissue culture flasks containing RPMI-1640 medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Grand Island) and antibiotics.

Drugs

Paclitaxel and SN-38 were provided by Brystol Myers Squibb Japan Co. (Tokyo), and Yakult Co. (Tokyo), respectively. Paclitaxel was dissolved in dimethyl sulfoxide (Sigma Chemical Co., St. Louis, Mo.). SN-38 was dissolved in 0.1 *N* NaOH. Drugs were diluted with RPMI-1640 plus 10% FBS. The final concentration of dimethyl sulfoxide in the medium was less than 0.1%, and it had no effect on cell growth inhibition in our experiments.

Cell growth inhibition by combined anticancer agents

On day 0, cells growing in the exponential phase were harvested with 0.05% trypsin and 0.02% EDTA, and resuspended to a final concentration of 5.0×10^3 cells/ml in fresh medium containing 10% FBS and antibiotics. Cell suspensions (100 μl) were dispensed using a multichannel pipette into the individual wells of a 96-well tissue culture plate with a lid (Falcon, Oxnard, Calif.). Each plate had one 8-well control column containing medium alone and one 8-well control column containing cells but no drug. Four plates were prepared for each drug combination. The cells were reincubated overnight to allow attachment.

Simultaneous exposure to paclitaxel and SN-38

After cell attachment, solutions of paclitaxel and SN-38 (50 μl) at different concentrations were added to individual wells (paclitaxel preceding SN-38 by about 10 min). The plates were incubated under the same conditions for 24 h. The cells were then washed twice with culture medium containing 1% FBS, and then fresh medium containing 10% FBS (200 μl) and antibiotics was added. The cells were incubated again for 4 days.

Sequential exposure to paclitaxel and SN-38

After cell attachment, medium containing 10% FBS (50 μl) and solutions of paclitaxel or SN-38 (50 μl) at different concentrations were added to individual wells. The plates were then incubated under the same conditions for 24 h. The cells were washed twice with culture medium containing 1% FBS, and then fresh medium containing 10% FBS (150 μl) and antibiotics was added, followed by the addition of solutions of SN-38 or paclitaxel (50 μl) at different concentrations. The plates were incubated again under the same conditions for 24 h. The cells were then washed twice with culture medium, and fresh medium containing 10% FBS (200 μl) and antibiotics was added. The cells were then incubated again for 3 days.

MTT assay

The viability of the cells was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazonium bromide (MTT) assay as described previously [12]. For all cell lines examined, we established a linear relationship between the MTT assay and the cell number within the range of the experiments shown.

Isobologram method

The dose-response interactions between paclitaxel and SN-38 at the point of IC_{80} were evaluated by the isobologram method of Steel and Peckham (Fig. 1) [37]. The IC_{80} was defined as the concentration of drug that produced 80% cell growth inhibition, i.e. 80% reduction in absorbance. We recently began using the IC_{80} value instead of the more common IC_{50} value, since, in previous experiments, the combined effects at the IC_{50} were sometimes different from those at the IC_{80}, which would be more relevant than the IC_{50} for cancer chemotherapy. Although the drug interaction at the IC_{90} or more would be more important than both the IC_{50} and the IC_{80}, it is difficult to get reliable data at the IC_{90} or more using the insensitive MTT assay.

When the dose-response curves are far from linear, as is usually the case in cancer chemotherapy and was the case in this study, the nature of an additive response is controversial [3, 7, 37]. The fractional product concept [40], the classical isobologram [21], the median effect principle [3], the isobologram method of Steel and Peckham [37] and many other methods [7] have been proposed for evaluating the effects of drug combinations. We have been using the Steel and Peckham isobologram method because it can cope with any agents with unclear cytotoxic mechanisms and any dose-response curves of anticancer agents. There is an area of uncertainty whose magnitude depends upon the nonlinearity of the responses. The extent of the uncertainty is best judged by the use of this isobologram, which is an isoeffect plot indicating the separate doses of two agents that in combination give the isoeffects [36].

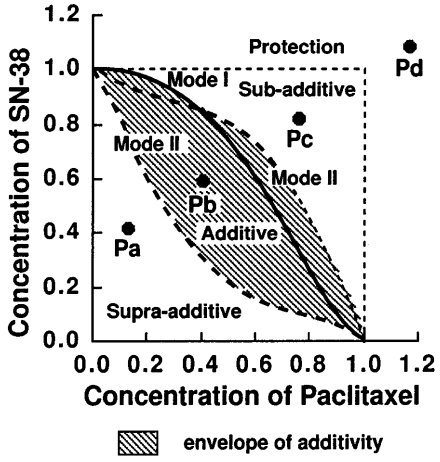


Fig. 1 Isobologram analysis (Steel and Peckham) of the dose-response interactions between paclitaxel and SN-38 at the IC_{80}. The envelope of additivity, surrounded by mode I (*solid line*) and mode II (*dotted lines*) isobologram lines, was constructed from the dose-response curves of paclitaxel alone and SN-38 alone. The concentrations which produced 80% cell growth inhibition (IC_{80}) are expressed as 1.0 in the ordinate and the abscissa of the isobologram. The data points *Pa*, *Pb*, *Pc*, and *Pd* indicate supraadditive, additive, subadditive, and protective effects, respectively

In the present study, the dose-response curves were plotted on a semilog scale as a percentage of the control, the cell number of which was obtained from the samples not exposed to the drugs administered simultaneously. The dose-response curves obtained by the simultaneous and sequential exposure to paclitaxel and SN-38 in WiDr cells are shown in Fig. 2. The paclitaxel concentrations are shown on the abscissa. Dose-response curves in which the SN-38 concentrations are shown on the abscissa can be made based on the same data (figure not shown). Based upon the dose-response curves of paclitaxel alone and SN-38 alone, three isoeffect curves (mode I and mode II lines) were constructed (Fig. 1). A French curve model fitted to the data was used to make dose-response curves and the isobolograms. The procedure for making the isobologram has been described in detail previously [13]. *Mode I line*: if the two agents act additively by independent mechanisms, the combined data points would lie near the mode I line (heteroaddition). *Mode II lines*: if the agents act additively by similar mechanisms, the combined data points would lie near the mode II lines (isoaddition). When both agents have the same dose-response curves, the mode II lines converge to make a straight line connecting the 1.0 points on the ordinate and abscissa. When both agents have a linear dose-response curve, the mode I and mode II lines converge to make a straight line connecting the 1.0 points on the ordinate and abscissa.

Since we cannot know in advance whether the combined effects of two agents will be heteroadditive, isoadditive, or intermediate between these extremes, all possibilities should be considered. Thus, when the data points of the drug combination fall within the area surrounded by mode I and/or mode II lines (i.e. within the envelope of additivity), the combination is described as additive. The envelope of additivity should not be regarded as a reliable definition of additivity. It is an expression of the uncertainty of this method and the concept of uncertainty is important in the use of the isobologram method of Steel and Peckham.

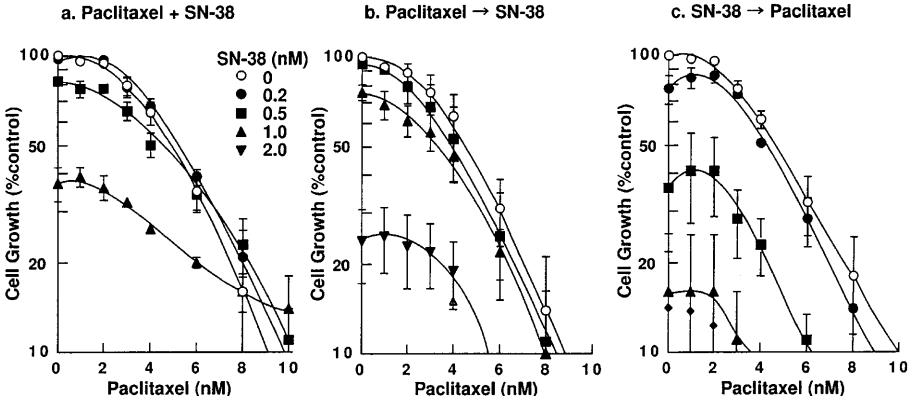
We used this envelope not only to evaluate the simultaneous administration of combinations of paclitaxel and SN-38, but also to evaluate the sequential combinations, since the cytotoxicity of the first agent could be modulated by the second agent under our experimental conditions.

A combination that gives data points to the left of the envelope of additivity (i.e. the combined effect is caused by lower doses of the two agents than is predicted) can confidently be described as supraadditive (synergism). A combination that gives data points to the right of the envelope of additivity, but within the square or on the line of the square can be described as subadditive (i.e. the combination is superior or equal to a single agent but is less than additive). A combination that gives data points outside the square can be described as protective (i.e. the combination is inferior in cytotoxic action to a single agent). A combination with both subadditive and/or protective interactions can confidently be described as antagonistic.

According to the fractional product concept, a combination that gives a data point on the mode I line is additive, and a combination that gives a data point to the left or right of the mode I line is synergistic or antagonistic, respectively. According to the classical isobologram method, a combination that gives a data point on a straight line connecting the 1.0 points on the ordinate and abscissa is additive, and a combination that gives a data point to the left or right of a straight line is synergistic or antagonistic, respectively.

The isobologram method of Steel and Peckham has the area of uncertainty (envelope of additivity), while many other approaches have an additive line. Therefore, the isobologram of Steel and Peckham is generally more strict regarding synergism and antagonism.

Fig. 2a–c Dose-response curves for paclitaxel alone, SN-38 alone, and their combinations in WiDr cells. Cells were exposed to drugs simultaneously for 24 h (**a**), paclitaxel first for 24 h followed by SN-38 for 24 h (**b**), and the reverse sequence (**c**). After 5 days, the cell numbers were determined using the MTT assay and were plotted as a percentage of the control (cells not exposed to drugs). The concentrations of SN-38 for each symbol are shown at the upper right of panel **a**. Paclitaxel concentrations are shown on the abscissa. Each point represents the mean value ± SE (*bars*) of at least three independent experiments performed in quadruplicate. The dose-response curves with SN-38 concentrations on the abscissa were made using the same dose-response data (not shown)



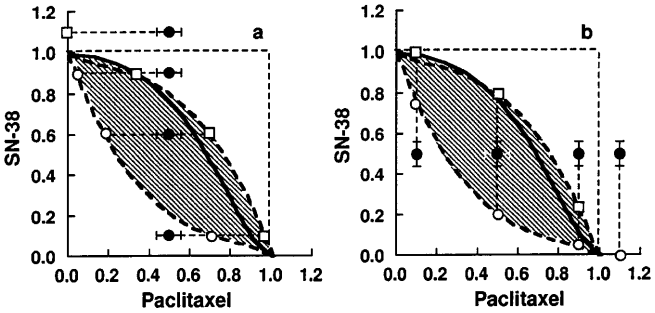


Fig. 3a,b Isobologram analysis of the observed data (●), the predicted minimum data (○) and the predicted maximum data (□) for an additive effect (**a** with horizontal error bars and **b** with vertical error bars). The data with horizontal and vertical error bars were obtained from the dose-response curves with paclitaxel concentrations on the abscissa (Fig. 2) and the dose-response curves with SN-38 concentrations on the abscissa (not shown), respectively. The predicted minimum or maximum data for an additive effect were on the borderline (mode I or mode II lines) between the additive and supraadditive areas, or between the additive and the subadditive (or protective) areas, respectively

Data analysis

The observed data were compared with the predicted maximum and minimum data for an additive effect (Fig. 3). If the mean value of the observed data was equal to or smaller than that of the predicted maximum data and equal to or larger than that of the predicted minimum data, the combination was regarded as additive. If the mean value of the observed data was smaller than that of the predicted minimum data or larger than that of the predicted maximum data, the combination was considered as synergistic or antagonistic, respectively. To determine whether the condition of synergism (or antagonism) truly existed, a statistical analysis was performed. Since the data did not follow a normal distribution, the Wilcoxon signed-ranks test was used for comparing the observed data with the predicted minimum (or maximum) data for an additive effect. Probability values (P) $\leqq 0.05$ were considered significant. Cases with $P > 0.05$ were regarded as being additive to synergistic (or additive to antagonistic). All statistical analyses were performed using the Stat View 4.01 software program (Abacus Concepts, Berkeley, Calif.).

Results

Figure 2 shows the dose-response curves of WiDr colon cancer cells to the combination of paclitaxel and SN-38 in various schedules: simultaneous exposure to the drugs, sequential exposure to paclitaxel followed by SN-38 and sequential exposure to SN-38 followed by paclitaxel. Each isobologram was generated based on such dose-response curves.

Simultaneous exposure to paclitaxel and SN-38

Figure 4 shows the isobolograms of the A549, MCF7, PA1, and WiDr cells after simultaneous exposure to paclitaxel and SN-38. The combined effects of simultaneous exposure to these drugs differed among the four cell lines. In the PA1 cells, the combined data points fell within the envelope of additivity (Fig. 4c). The mean value of the data (0.73) was larger than that of the

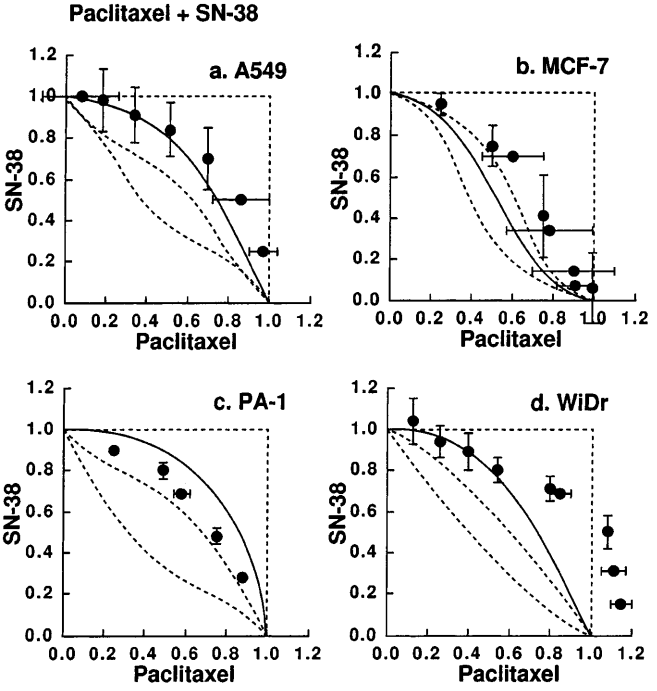


Fig. 4a–d Isobolograms of simultaneous exposure to paclitaxel and SN-38 in A549 (**a**), MCF7 (**b**), PA1 (**c**), and WiDr (**d**) cells. The data are the mean values ± SE (*bars*) of at least three independent experiments. The data points for the combination fell in the area of subadditivity for the A549 and MCF7 cells, and in the areas of additivity, subadditivity and protection for the WiDr cells, suggesting antagonistic interactions, and within the envelope of additivity for the PA1 cells, suggesting an additive interaction

predicted minimum data (0.35) and smaller than that of the predicted maximum data for an additive effect (0.86) (Table 1), indicating that simultaneous exposure to the two agents produced an additive effect. In the A549, MCF7 and WiDr cells, the combined data points fell mainly in the area of subadditivity and/or protection (Fig. 4a,b,d), and the mean values of the observed data (0.77, 0.67, and 0.89) were larger than those of the predicted maximum additive data (0.73, 0.59, and 0.73, respectively; Table 1). The observed data and the predicted maximum data were compared using the Wilcoxon signed-ranks test (Fig. 5). The observed data in the A549, MCF, and WiDr cells were significantly higher than the predicted maximum data ($P < 0.05$, $P < 0.02$, and $P < 0.05$, respectively), indicating antagonistic effects on these three cell lines of simultaneous exposure to these two drugs.

Sequential exposure to paclitaxel first followed by SN-38

Figure 6 shows representative isobolograms of A549, MCF7, PA1, and WiDr cells exposed first to paclitaxel and then to SN-38. In this experiment, similar combined effects were shown in all four cell lines; most of the combined data points fell within the envelope of

Table 1 Mean values of observed, predicted minimum, and predicted maximum data

Schedule	Cell line	Observed data	Predicted minimum[a]	Predicted maximum[b]	Effect
Paclitaxel + SN-38	A549	0.77	0.44	0.73	Antagonism ($P < 0.05$)
	MCF7	0.67	0.43	0.59	Antagonism ($P < 0.02$)
	PA1	0.73	0.35	0.86	Additive
	WiDr	0.89	0.47	0.73	Antagonism ($P < 0.05$)
Paclitaxel → SN-38	A549	0.75	0.27	0.83	Additive
	MCF7	0.82	0.49	0.83	Additive
	PA1	0.64	0.26	0.74	Additive
	WiDr	0.8	0.46	0.8	Additive
SN-38 → Paclitaxel	A549	0.84	0.49	0.85	Additive
	MCF7	0.66	0.51	0.73	Additive
	PA1	0.56	0.4	0.69	Additive
	WiDr	0.68	0.42	0.7	Additive

[a] Predicted minimum data for an additive effect
[b] Predicted maximum data for an additive effect

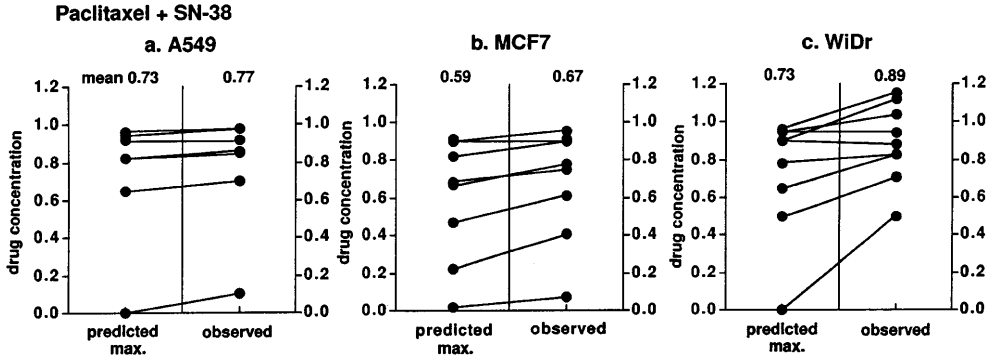


Fig. 5a–c Comparison of the predicted maximum data for an additive effect with the observed data obtained from simultaneous exposure to paclitaxel and SN-38 for A549 (**a**), MCF7 (**b**), and WiDr (**c**) cells. The observed data were significantly higher than the predicted maximum data ($P < 0.05$, $P < 0.02$, and $P < 0.05$, respectively) by Wilcoxon signed-ranks test, indicating antagonistic effects of simultaneous exposure to this combination

additivity. The mean value of the data was smaller than that of the predicted maximum data and larger than that of the predicted minimum data in all four cell lines (Table 1). These findings suggest that sequential exposure to paclitaxel first followed by SN-38 produced additive effects.

Sequential exposure to SN-38 first followed by paclitaxel

Figure 7 shows representative isobolograms of the four cell lines treated with the reverse sequence (SN-38 then paclitaxel). Similar combined effects were shown in all four cell lines; most of the combined data points fell within the envelope of additivity. The mean value of the data was smaller than that of the predicted maximum data and larger than that of the predicted minimum data in all cell lines (Table 1). These findings suggest that sequential exposure to paclitaxel first followed by SN-38 also produced additive effects.

There was no difference in the drug interaction at the IC_{80} and IC_{50} levels (data not shown) in this combination study.

Discussion

Scheduling effects often play a significant role in the outcome of treatment and therefore require careful consideration in the design of combination protocols. Clinical combination studies of paclitaxel with cisplatin and with doxorubicin have demonstrated that the schedule of the administration of these drugs is an important variable that must be considered to maximize the therapeutic index [2, 32]. In preclinical studies, a schedule-dependent interaction has been observed for paclitaxel in combination with cisplatin, methotrexate, doxorubicin, and 5-fluorouracil [1, 10, 14, 15, 19, 32, 41].

In the present study, we investigated the optimal schedule for combination chemotherapy with paclitaxel and SN-38 (the active metabolite of irinotecan) in four human cancer cell lines in culture. The effects of drug–drug interactions were evaluated using the isobologram method of Steel and Peckham [37]. We found that the cytotoxic interaction between paclitaxel and SN-38 was

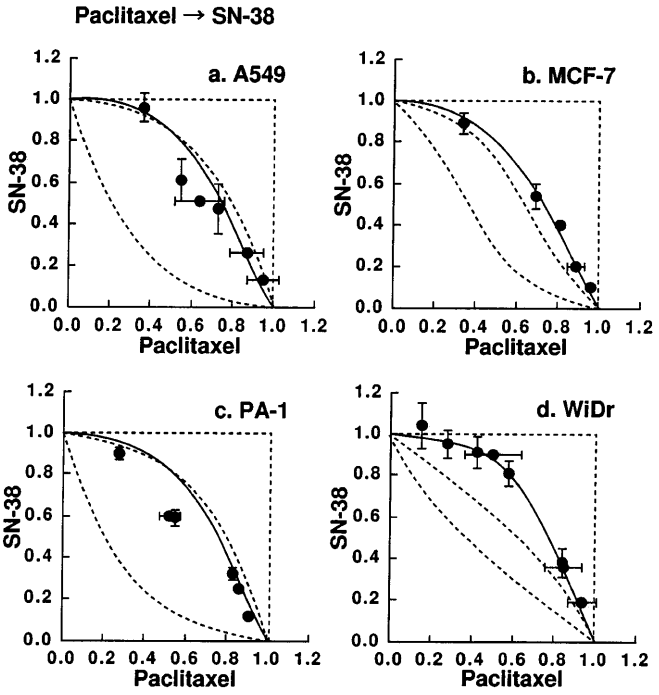


Fig. 6a–d Isobolograms of sequential exposure to paclitaxel followed by SN-38 in A549 (**a**), MCF7 (**b**), PA1 (**c**), and WiDr (**d**) cells. The data are the mean values ± SE (*bars*) of at least three independent experiments. The data points for the combination fell within the envelope of additivity in all four cell lines, suggesting additive interactions

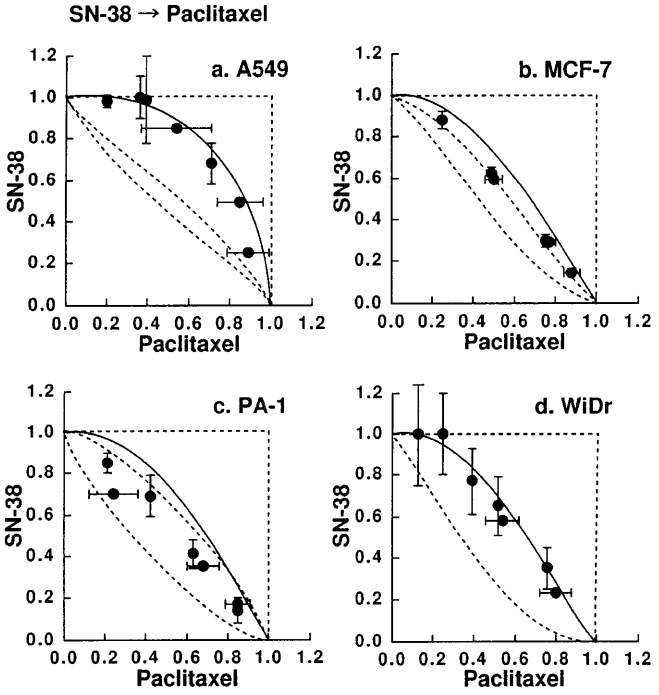


Fig. 7a–d Isobolograms of sequential exposure to SN-38 followed by paclitaxel in A549 (**a**), MCF7 (**b**), PA1 (**c**), and WiDr (**d**) cells. The data are the mean values ± SE (*bars*) of at least three independent experiments. The data points for the combination were mainly within the envelope of additivity for all four cell lines, suggesting additive interactions

schedule- and cell line-dependent. Simultaneous exposure to paclitaxel and SN-38 had additive effects in PA1 cells, while the same schedule had antagonistic effects in A549, MCF7, and WiDr cells. A simultaneous administration schedule is an attractive model for a clinical regimen, and, in general, anticancer agents in combination therapy are administered simultaneously. However, our present findings suggest that the simultaneous administration of paclitaxel and irinotecan may be less effective than anticipated.

Although there are no experimental data on the use of the combination of paclitaxel and irinotecan (or SN-38), Chou et al. [3] and Kaufmann et al. [16] reported in vitro experimental data on the combination of paclitaxel and another topo-I inhibitor, topotecan, using the median effect principle. Chou et al. reported that simultaneous exposure to paclitaxel and topotecan for 4 days produced synergistic effects against the human teratocarcinoma 833 K and 833/64CP10 cell lines [3], while Kaufmann et al. reported that the simultaneous exposure to these two agents for 7–8 days produced antagonistic effects against human lung cancer A549 cells [16]. Our data are consistent with those of Kaufmann et al.

Sequential exposure to paclitaxel followed by SN-38, and the reverse sequence, had additive effects in all four carcinoma cell lines; no schedule had supraadditive (synergistic) effects with this combination. Generally, combinations of drugs with a synergistic or additive interaction, but not those with an antagonistic interaction, are incorporated into clinical studies. The present data suggest that the optimal schedule for this combination may be the sequential administration of paclitaxel followed by irinotecan, or the reverse sequence, and not the simultaneous administration of these agents.

When considering the potential implications of these results, several limitations of the study must be kept in mind. First, the additive effects of a combination do not indicate a therapeutic advantage if the combination is synergistic for critical normal tissues. Since both paclitaxel and irinotecan have dose-limiting bone marrow toxicity, this toxicity must be considered, as should unexpected toxicities in clinical combination studies. Second, the monolayer cell culture system cannot reproduce the cellular interaction occurring at the three-dimensional tumor level. Third, the results of an in vitro study, which used a constant level of drug exposure, and clearly could not provide data on the pharmacokinetic behavior and tissue distribution of the two agents examined, cannot easily be extrapolated to patients. The administration of irinotecan before paclitaxel in a clinical setting is not necessarily a truly sequential protocol, since SN-38 has a long half-life [33] and could be present in the plasma during treatment with paclitaxel. In addition, both agents bind strongly to tissues, and sequential administration may therefore be tantamount

to simultaneous administration at the tissue level. Fourth, in vitro experiments cannot measure pharmacokinetic interactions. Results of clinical combination studies of paclitaxel and cisplatin or doxorubicin suggest that the pharmacokinetic interaction has an influence on the toxicity and clinical efficacy of these combinations [2, 32]. These findings also suggest the importance of in vivo studies of paclitaxel in combinations.

The mechanism underlying the antagonistic interaction that occurs with the simultaneous exposure of cells to paclitaxel and SN-38 is unclear. Both agents are believed to induce cell cycle-dependent effects and to delay cell-cycle traverse in specific phases of the cycle. Paclitaxel has a cytotoxic effect by blocking cells in G_2/M (22, 31), while irinotecan has major cytotoxic effects on cells in the S phase and accumulates in cells in the late S and G_2 phases [4, 28]. Thus, one agent might reduce the cytotoxicity of the other by preventing cells from entering the specific phase in which the cells are most sensitive to the other agent. Sequential exposure to the two agents produced additive effects in the present study, but not antagonistic effects. Since cells escape the blocks and enter the next round of DNA replication after the withdrawal of paclitaxel [22] and irinotecan [28], this may explain the additive but not antagonistic effects between these agents using sequential administration. However, the elucidation of the specific mechanisms associated with the additive or antagonistic interactions of this combination remains complicated by the fact that our present knowledge of the precise ways in which paclitaxel and SN-38 actually exert their cytotoxic effects on cells is still limited. Further studies of the changes in the biochemical pharmacology of this combination are required to clarify the cytotoxic interactions between paclitaxel and SN-38 in various schedules.

In conclusion, antagonistic effects were observed in three of the four human cancer cell lines examined when they were exposed to paclitaxel and SN-38 simultaneously, whereas additive effects were observed in all four of the cell lines when they were sequentially exposed to paclitaxel followed by SN-38, and to the reverse sequence. These findings suggest that the simultaneous administration of paclitaxel and irinotecan may be inadequate, and that the optimal schedule of this combination may be by sequential administration. Continued preclinical and clinical studies should provide further insights and assist in determining the optimal schedule of this combination for clinical use.

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