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Yasuhiko Kano · Miyuki Akutsu · Kenichi Suzuki
Kiyoshi Mori · Yasuo Yazawa · Saburo Tsunoda

Schedule-dependent interactions between paclitaxel and etoposide in human carcinoma cell lines in vitro

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Abstract Clinical studies of paclitaxel in combination with etoposide against solid tumors have been carried out. The combination schedules used in these studies are different. We studied the cytotoxic effects of paclitaxel with etoposide against four human cancer cell lines in vitro to determine the optimal schedule of this combination at the cellular level. Cells were exposed simultaneously to paclitaxel and to etoposide for 24 h or sequentially to one drug for 24 h followed by the other for 24 h, after which they were incubated in drug-free medium for 4 and 3 days, respectively. Cell growth inhibition was determined by an MTT reduction assay. The effects of drug combinations at concentrations producing 80% inhibition (IC_{80}) were analyzed by the isobologram method of Steel and Peckham. The cytotoxic effect of paclitaxel and etoposide was cell line- and schedule-dependent. Simultaneous exposure to paclitaxel and etoposide for 24 h produced additive effects in the lung cancer cell line A549 and ovarian cancer PA1 cells, and antagonistic effects in the breast cancer cell line MCF7 and colon cancer WIDr cells. Sequential exposures to paclitaxel followed by etoposide and vice versa produced additive effects in all four cell lines. These results suggest that maximum cytotoxic effects can be obtained with sequential administration, but not simultaneous administration, of paclitaxel and etoposide. These findings may have important clinical implications for this combination.

Key words Paclitaxel · Etoposide · Drug combination · Isobologram

Y. Kano (✉) · M. Akutsu · K. Mori · S. Tsunoda
Division of Medical Oncology,
Tochigi Cancer Center,
Yonan, 4-9-13, Utsunomiya,
Tochigi, 320-0834, Japan
e-mail: ykano@tcc.pref.tochigi.jp
Tel.: +81-(0)28-658-5151;
Fax: +81-(0)28-658-5488

K. Suzuki
Division of Laboratory Medicine,
Tochigi Cancer Center,
Utsunomiya, Tochigi, 320-0834, Japan

Y. Yazawa
Division of Orthopedic Oncology,
Tochigi Cancer Center,
Utsunomiya, Tochigi, 320-0834, Japan

Introduction

Paclitaxel is a new antimicrotubular agent with significant activity against a variety of solid tumors, including lung cancer, breast cancer, and ovarian cancer [2, 10, 12, 24, 38]. Unlike vinca alkaloids, this drug promotes the polymerization and stabilization of tubulin to microtubules, thereby inhibiting the dynamic reorganization of the microtubule network required for mitosis and cell division [31, 46, 47]. Cells in the S phase are most sensitive to paclitaxel and accumulate in the G_2/M phase [37]. The primary dose-limiting toxicity of paclitaxel is myelotoxicity, mainly neutropenia. Mild peripheral neuropathy has been reported, and hypersensitivity reactions and cardiac arrhythmia have been rarely observed [11, 19, 51].

Etoposide is a semisynthetic podophyllotoxin derivative with a broad spectrum of activity against a wide variety of malignancies, including leukemia, lymphoma, germinal tumor, and lung cancer [44]. Etoposide is thought to exert its main cytotoxic action by stabilizing cleavable complexes formed by DNA and the nuclear enzyme topoisomerase II [34]. Cells in the S phase are most sensitive to etoposide, and etoposide-treated cells accumulate in the late S/G_2 phase [35]. The dose-limiting toxicity of etoposide is myelotoxicity, mainly neutropenia. Nonhematological toxicities are mild to moderate. Despite its use as a single agent and in a number of combination chemotherapy regimens, the optimal use of etoposide remains unknown.

The combination of paclitaxel and etoposide is rational, based on the marked antitumor activity of both agents against a variety of solid tumors, their different

cytotoxic mechanisms, and the different toxic profiles other than that of neutropenia. There has been interest in identifying the optimal schedules for this combination, but considerable controversy exists over whether these drugs produce synergistic, additive, or antagonistic effects when given together or sequentially [7, 9, 14, 20, 30, 40, 50]. The combination of paclitaxel and etoposide in various schedules has been used for the treatment of lung cancer, breast cancer, colon cancer and a variety of other cancers [5, 6, 18, 33, 41, 43, 49]. We therefore conducted an in vitro investigation of the effects of paclitaxel and etoposide in combination against human cancer cell lines in various schedules. The analysis of the effects of the drug-drug interactions was carried out by the isobologram method proposed by Steel and Peckham [48].

Materials and methods

Cell lines

The experiments were conducted with four human carcinoma cell lines: the non-small-cell lung cancer cell line A549, the breast cancer cell line MCF7, the ovarian cancer cell line PA1, and the colon cancer cell line WiDr. These cells were obtained from the American Type Culture Collection (Rockville, Md.) and maintained in 25-cm^2 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 Biological Co.) and antibiotics. The cells used were free of mycoplasma infection. A549, MCF7, and PA1 cells were blc-2-positive.

Drugs

Paclitaxel and etoposide were provided by Bristol-Myers Squibb Japan Co. (Tokyo) and Nihon Kayaku Co. (Tokyo), respectively. Paclitaxel was dissolved in dimethyl sulfoxide (Sigma Chemical Co., St Louis, Mo.), and etoposide was dissolved in RPMI-1640. The drugs were diluted with culture medium. 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 study. Since the cytotoxic levels of paclitaxel and etoposide in clinical practice are generally maintained for more than 10 h, a 24-h exposure to paclitaxel and etoposide was used in the present experiments.

Inhibition of cell growth by combinations of anticancer agents

Exponentially growing cells were harvested with trypsin (0.05%)/EDTA (0.02%) and resuspended to a final concentration of 5.0×10^3 cells/ml in fresh medium containing 10% FBS and antibiotics. Aliquots of the 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 eight-well control column containing medium alone and one eight-well control column containing cells but no drug. Four plates were prepared for each drug combination schedule in each cell line. The cells were reincubated overnight in a humidified atmosphere containing 5% CO_2 at 37 °C to allow attachment.

Simultaneous exposure to paclitaxel and etoposide

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

Sequential exposure to paclitaxel first followed by etoposide and vice versa

After cell attachment, aliquots of medium containing 10% FBS (50 μl) and solutions of paclitaxel (or etoposide) (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 fresh medium containing 10% FBS (150 μl) and antibiotics, and aliquots of etoposide (or paclitaxel) solution (50 μl) at different concentrations were added. The plates were incubated again under the same conditions for 24 h. After treatment, the cells were washed twice, and fresh medium (200 μl) was provided. The cells were then incubated again for 3 days.

MTT assay

Viable cell growth was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously [27]. For all cell lines examined, we were able to establish a linear relationship between the MTT assay and the cell number within the range used in these experiments.

Isobologram method

The dose-response interactions between paclitaxel and etoposide at the point of IC_{80} were evaluated by the isobologram method of Steel and Peckham [48]. The theoretical basis of the isobologram method and the procedure for making isobolograms have been described in detail previously [26, 28, 48].

Based upon the dose-response curves of paclitaxel and etoposide, three isoeffect curves were constructed (Fig. 1). If the agents

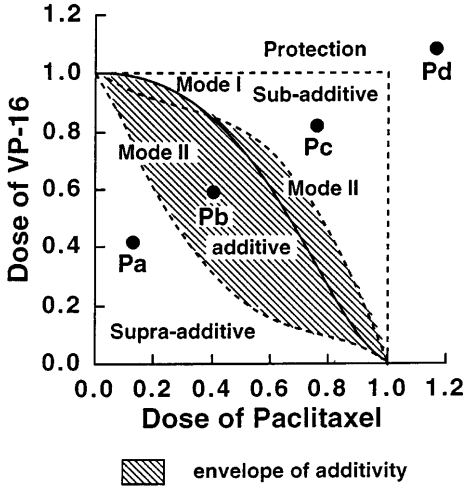


Fig. 1 Isobologram analysis (Steel and Peckham) of the dose-response interactions between paclitaxel and etoposide (VP-16). The envelope of additivity, surrounded by mode I (*solid*) and mode II (*dotted*) isobologram lines, was constructed from the dose-response curves of paclitaxel alone and etoposide (VP-16) alone. The concentrations that produced 80% cell growth inhibition are expressed as 1.0 on the ordinate and the abscissa of the isobologram. The data points *Pa*, *Pb*, *Pc*, and *Pd* show supraadditive, additive, subadditive, and protective effects, respectively

are acting additively by independent mechanisms, combined data points would lie near the mode I line (heteroaddition). If the agents are acting additively by similar mechanisms, combined data points would lie near the mode II lines (isoaddition).

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 fell within the area surrounded by three lines (envelope of additivity), the combination was regarded as additive. The envelope of additivity should not be considered as a reliable definition of additivity. The expression of the uncertainty is an important concept in the isobologram method of Steel and Peckham.

We used this envelope not only to evaluate combinations in which cells were simultaneously exposed to paclitaxel and etoposide, but also to evaluate combinations in which the cells were sequentially exposed to both agents, since the cytotoxicity of the first agent could be modulated by the second agent under our experimental conditions.

When the data points fell to the left of the envelope (i.e. the combined effect was caused by lower doses of the two agents than was predicted), we regarded the drug combination as having a supraadditive effect (synergism). When the points fell to the right of the envelope (i.e. the combined effect was caused by higher doses of the two agents than was predicted), but within the square or on the line of the square, we regarded the combination as having a subadditive effect, i.e. the combination was superior or equal to a single agent but was less than additive. When the data points were outside the square, the combination was regarded as having a protective effect, i.e. the combination was inferior in cytotoxic action to a single agent. Both subadditive and protective interactions were regarded as antagonism.

Simultaneous exposure and sequential exposure to paclitaxel with itself produced additive effects (data not shown). Self-synergism and self-antagonism were not produced.

Data analysis

Data were analyzed as described previously [29]. When the observed data points in combination fell mainly within the envelope of additivity, the combination was considered as having an additive effect. The mean value of the observed data was compared with that of the predicted maximum values and that of the predicted minimum values for an additive effect, which were on the border line (mode I or mode II lines) between the additive area and subadditive area or supraadditive area. If the mean value of the observed data was equal to or smaller than that of the predicted maximum values and equal to or larger than that of the predicted minimum values, the combination was regarded as having an additive effect.

When the observed data points of a combination fell mainly in the area of supraadditivity or in the areas of subadditivity and protection, and the mean value of the observed data was smaller than that of the predicted minimum values or larger than that of the predicted maximum values, the combination was considered to have a synergistic or antagonistic effect, respectively. To determine whether the condition of synergism (or antagonism) truly existed, a Wilcoxon's signed-ranks test was performed for comparing the observed data with the predicted minimum (or maximum) values for an additive effect. Probability (P) values $\leqq 0.05$ were considered significant. Combinations with $P > 0.05$ were regarded as having an additive to synergistic (or an additive to antagonistic) effect. All statistical analyses were performed using the Stat View 4.01 program (Abacus Concepts, Berkeley, Calif.).

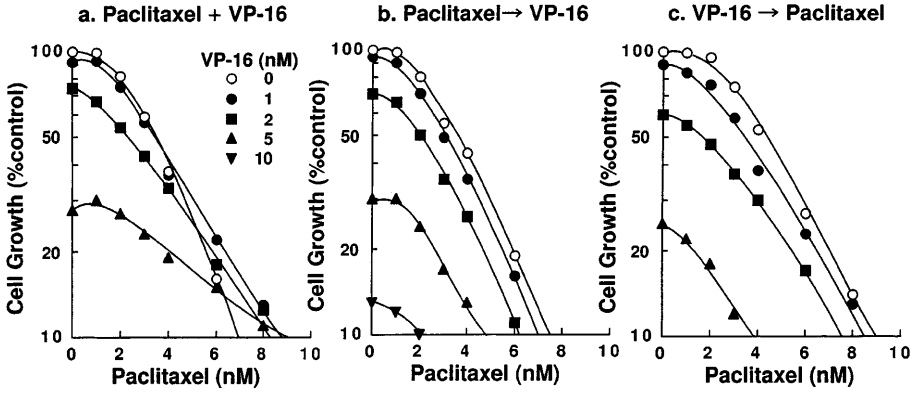
Results

Figure 2 shows the dose-response curves from the exposure of WiDr cells to paclitaxel and etoposide for 24 h in various schedules: simultaneous exposure to the drugs, sequential exposure to paclitaxel followed by etoposide, and sequential exposure to etoposide followed by paclitaxel. Isobolograms at the IC_{80} were generated based upon these dose-response curves.

Simultaneous exposure to paclitaxel and etoposide

Figure 3 shows the isobolograms of the A549, MCF7, PA1, and WiDr cells exposed to paclitaxel and etoposide simultaneously. The combined effects of the simultaneous exposure to drugs were different depending upon the cell line used. For the A549, and PA1 cells, the combined data points fell within the envelope of additivity. The mean values of the observed data (0.70 and 0.69, respectively) were larger than those of the predicted minimum values (0.45 and 0.34) and smaller than those of the predicted maximum values for an additive effect (0.75 and 0.73, respectively; Table 1), indicating additive effects.

Fig. 2a–c Dose-response curves for paclitaxel alone, etoposide (VP-16) alone, and their combinations in WiDr cells. Cells were exposed simultaneously to the two drugs for 24 h (**a**), sequentially to paclitaxel (24 h) followed by etoposide (24 h) (**b**), and sequentially to etoposide (24 h) followed by paclitaxel (24 h) (**c**). Each assay was run in quadruplicate; cell numbers were measured using the MTT assay after 5 days and are plotted as percentage of the control (cells not exposed to drugs). Data are the mean values from for at least three independent experiments. Paclitaxel concentrations are shown on the abscissa. The etoposide concentrations are indicated by the symbols as shown on the upper right of **a**



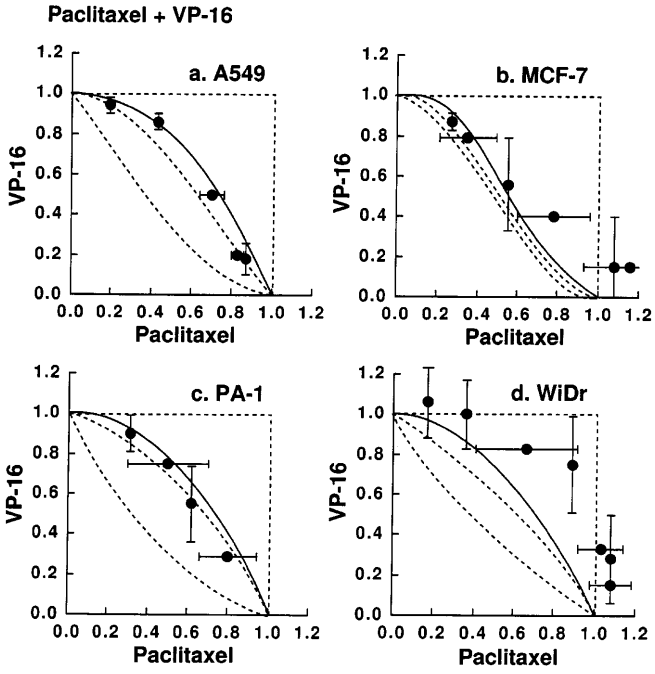


Fig. 3a–d Isobolograms of simultaneous exposure to paclitaxel and etoposide (VP-16) of A549 (**a**), MCF7 (**b**), PA1 (**c**), and WiDr (**d**) cells. Data are mean values ±SE (*bars*) from at least three independent experiments. For the A549, and PA1 cells, all or most of the data points of the combinations fall within the envelope of additivity. For the MCF7 cells, the data points fall within the envelope of additivity, and areas of subadditivity and protection. For the WiDr cells, the data points fall in the areas of subadditivity and protection

For the MCF7 and WiDr cells, the combined data points fell mainly in the area of subadditivity and/or protection, and the mean values of the observed data (0.65, and 0.84, respectively) were larger than those of the predicted maximum additive values (0.55, and 0.60, respectively; Table 1). The observed data and the predicted maximum values were compared using Wilcoxon's signed-ranks test. The observed data for the MCF7, and WiDr cells were significantly higher than the predicted maximum values ($P < 0.05$ and $P < 0.05$, respectively), indicating that the combination produced antagonistic effects.

Sequential exposure to paclitaxel followed by etoposide

Figure 4 shows the isobolograms of the four cell lines exposed first to paclitaxel and then to etoposide. Under these experimental conditions, the combined effects were similar in all four cell lines; all or most of the combined data points fell within the envelope of additivity. The mean values were smaller than the predicted maximum values and larger than the predicted minimum values for all four cell lines (Table 1). These results indicate that sequential exposure to paclitaxel followed by etoposide produced additive effects.

Sequential exposure to etoposide followed by paclitaxel

Figure 5 shows the isobolograms of cell lines exposed first to etoposide and then to paclitaxel. Under these experimental conditions, the combined effects were similar in all cell lines; all or most of the combined data points fell within the envelope of additivity. The mean values of the observed data were smaller than those of the predicted maximum values and larger than those of the predicted minimum values for all four cell lines (Table 1). These results indicate that sequential exposure to etoposide followed by paclitaxel produced additive effects.

Discussion

Clinical studies have shown that paclitaxel has promising activity against a variety of cancers and may result in major advances in cancer chemotherapy. Studies of

Table 1 Mean values of observed data, predicted minimum, and predicted maximum values for paclitaxel (*P*) in combination with etoposide (*E*)

Schedule	Cell line	Observed data	Predicted minimum[a]	Predicted maximum[b]	Effect
P + E	A549	0.70	0.45	0.75	Additive
	MCF7	0.65	0.44	0.55	Antagonism ($P < 0.05$)
	PA1	0.69	0.34	0.73	Additive
	WiDr	0.84	0.40	0.60	Antagonism ($P < 0.05$)
P → E	A549	0.65	0.50	0.71	Additive
	MCF7	0.47	0.38	0.64	Additive
	PA1	0.70	0.31	0.77	Additive
	WiDr	0.69	0.54	0.73	Additive
E → P	A549	0.52	0.17	0.78	Additive
	MCF7	0.54	0.23	0.58	Additive
	PA1	0.60	0.40	0.60	Additive
	WiDr	0.60	0.38	0.70	Additive

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

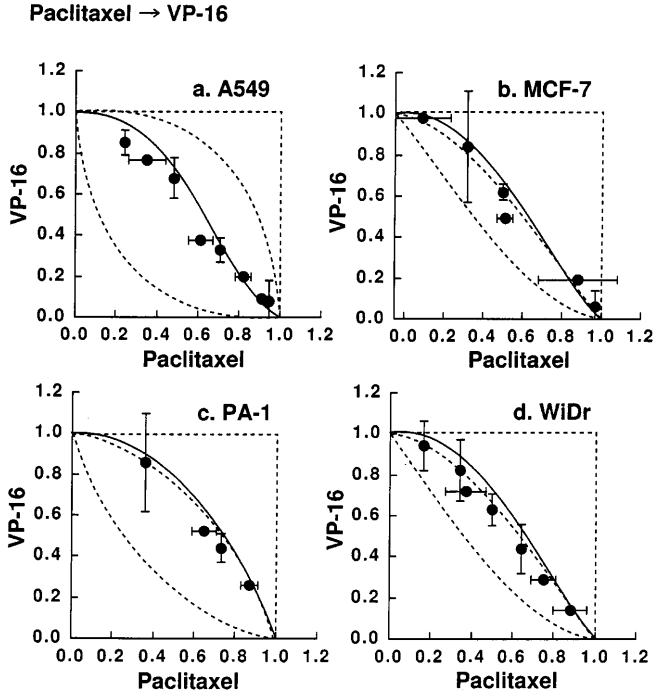


Fig. 4a–d Isobolograms of sequential exposure to paclitaxel followed by etoposide (VP-16) of A549 (**a**), MCF7 (**b**), PA1 (**c**), and WiDr (**d**) cells. Data are mean values ±SE (*bars*) from at least three independent experiments. For all four cell lines, all or most of the data points for the combination fall within the envelope of additivity

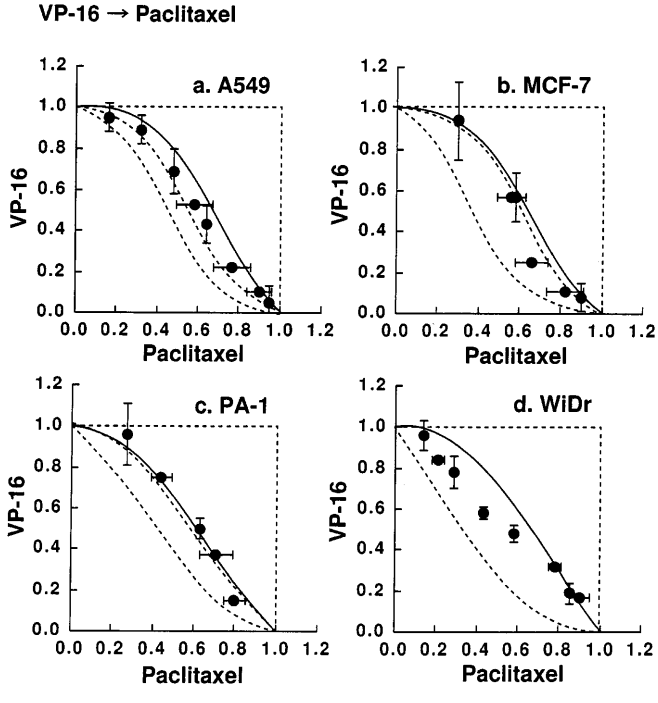


Fig. 5a–d Isobolograms of sequential exposure to etoposide (VP-16) followed by paclitaxel of A549 (**a**), MCF7 (**b**), PA1 (**c**), and WiDr (**d**) cells. Data are mean values ±SE (*bars*) from at least three independent experiments. For all four cell lines, all or most of the data points for the combination fall within the envelope of additivity

combination chemotherapy regimens of paclitaxel with a variety of anticancer agents including etoposide against solid tumors have been carried out. The purpose of the present study was to investigate the optimal schedule for paclitaxel in combination with etoposide against four human carcinoma cell lines at the cellular level.

We observed that simultaneous exposure to paclitaxel and etoposide produced antagonistic effects in MCF7 and WiDr cells at the IC_{80} level, but produced additive effects in A549 and PA1 cells. Sequential exposure to paclitaxel followed by etoposide and vice versa produced additive effects in all four cell lines. Nearly the same combined effects were observed at the IC_{50} level in all four cell lines (data not shown). These findings suggest that simultaneous administration is inadequate for this combination of agents and that the optimal schedule of paclitaxel and etoposide is sequential administration of paclitaxel followed by etoposide or vice versa. The cause of the schedule-dependence of these effects is unknown.

Previous in vitro studies of the interaction between paclitaxel and etoposide have shown conflicting results (Table 2). Chou et al. and Klaassen et al. found that simultaneous exposure to paclitaxel and etoposide produces synergistic effects [9, 30], while Frey et al., Budman et al., Viallet et al. and Perez and Buckwalter found that it produces antagonistic effects [7, 14, 40, 50]. Our data support the findings of the latter group. Hahn et al. and Viallet et al. observed that paclitaxel followed by etoposide in the presence of paclitaxel had antagonistic effects [20, 50]. This treatment schedule would result in not only sequential but also simultaneous exposure to these two agents. The simultaneous exposure might have acted antagonistically to influence the results.

Budman et al., Klaassen et al. and Perez and Buck walter studied the effects of a sequential exposure to these two agents, and their results also differed (Table 2) [7, 30, 40]. Regarding the paclitaxel/etoposide sequence, Klaassen et al. and Perez and Buckwalter found that it produces additive to synergistic effects [30, 40], while Budman et al. found that it produces antagonistic effects [7]. Regarding the etoposide/paclitaxel sequence, Budman et al. and Klaassen et al. found that it produces antagonistic effects [7, 30], while Perez and Buckwalter found that it produces synergistic effects [40]. These findings are also different from ours. We observed no difference between the effects of the paclitaxel/etoposide sequence and those of the etoposide/paclitaxel sequence, both of which produced additive effects.

These divergent preclinical data make selection of an optimal dose schedule for clinical trials of paclitaxel/etoposide combinations difficult. Differences in the experimental conditions such as the cell lines used, culture schedules, duration of drug exposure, and assay method used to determine viable cells might have produced the different results (Table 2). In addition, the methods for evaluating the effects of the drug combinations were different in each study (Table 2). Even if the same data were to be used for the evaluation, the methods have

Table 2 Experimental data for the combination of paclitaxel (*P*) and etoposide (*E*)

Reference	Cell line	Schedule (exposure time)	Interval	Analysis	Effect
20	A549, MCF7	P (24 h) → P + E (1 h)	0 h	Fractional product concept	Antagonism
9	833K/64CP10	P + E (96 h)		Median effect principle	Synergism
14	NLC-H123, N417, H1522	P + E		Three-dimensional model	Antagonism (2/3)
7	DU145, PC3, LnCap,	P + E (24 h)		Median effect principle	Antagonism (4/5)
	MCF7WT, MCF7/ADR	P (24 h) → E (24 h) ?	0 h ?		Antagonism (3/5)
		E (24 h) → P (24 h) ?	0 h ?		Antagonism (3/5)
30	A2780, A2780CP-2	P + E (2 h)		Classical isobologram	Synergism (3/4)
	TR170, TR170/731	P (2 h) → E (2 h)	22 h		Synergism (2/4)
		E (2 h) → P (2 h)	22 h		Antagonism (4/4)
50	NCI-H125, -H157, -H226, NCI-H358, -H661	E + P (120 h)		Isobologram (Steel and Peckham)	Antagonism (4/5)
	NCI-H226	P (24 h) → P + E (96 h)	0 h		Antagonism
40	A549, MCF7, MDA-231	P + E (24 h)		Median effect principle	Antagonism (2/3)
		P (24 h) → E (24 h)	0 h, 24 h		Synergism (3/3)
		E (24 h) → P (24 h)	0 h, 24 h		Synergism (3/3)
Present study	A549, MCF7, PA1, WiDr	P + E (24 h)		Isobologram (Steel and Peckham)	Antagonism (2/4)
		P (24 h) → E (24 h)	0 h		Additive (4/4)
		E (24 h) → P (24 h)	0 h		Additive (4/4)

often led to different conclusions [3, 13, 17, 42]. We used the isobologram method of Steel and Peckham. Although a large number of data are required, this method can be used to calculate the additive interaction of any combination, irrespective of the shapes of the dose-response curves of the agents and of whether they produce independent or overlapping damage. In general, the isobologram method of Steel and Peckham is stricter for synergism and antagonism than other methods for evaluating drug combinations and shows higher reproducibility of the results. Considering these factors, our findings would be consistent with the finding of Perez and Buckwalter [40] that the sequential administration but not the simultaneous administration of paclitaxel and etoposide is the optimal schedule of this combination.

Phase I and phase II studies of paclitaxel in combination with etoposide have been carried out [5, 6, 18, 31, 38, 40, 47]. The schedules used for this combination have been variable: the simultaneous administration of paclitaxel and etoposide [5, 6, 43], the sequential administration of paclitaxel followed by etoposide [49], and the reverse sequence [18, 33, 41, 43]. Green et al. have reported that the etoposide/paclitaxel sequence is tolerable but shows significant myelosuppression [18]. Boyer et al. compared schedules of the administration of oral etoposide (days 1 to 5) with paclitaxel given on day 1 or day 5 and recommend the former schedule as being less toxic [5]. Perez et al. have reported that the etoposide/paclitaxel sequence is feasible and toxicities are manageable [41]. Rosell et al. observed that the combination of paclitaxel and etoposide possesses substantial antitumor activity without significant toxicity in the etoposide (day 1 to 3)/paclitaxel (day 4) sequence as compared with the simultaneous/sequential administration of etoposide (day 1 to 3)/paclitaxel (day 1) [43]. The clinical findings of Rosell et al. and the preclinical findings of Perez et al. and the present study suggest that the sequential administration of paclitaxel and etoposide is the optimal schedule for this combination. A randomized phase II trial of paclitaxel followed by etoposide versus etoposide followed by paclitaxel would be the best clinical method for determining the optimal therapeutic index (therapeutic efficacy/toxicity).

Clinical studies of paclitaxel and etoposide used with other agents (mainly platinum derivatives) in various schedules have also been carried out [4, 8, 13, 15, 22, 39]. In most cases, paclitaxel has been administered simultaneously with etoposide and platinum derivatives. Although the simultaneous administration of cisplatin and etoposide is active and has been widely used in the treatment of a variety of cancers [1, 16, 21, 32, 52], preclinical and clinical studies of paclitaxel in combination with cisplatin have shown that the paclitaxel/cisplatin sequence is the optimal schedule, based on cytotoxic and pharmacokinetic interactions [45]. Considering these data and our findings, paclitaxel followed by etoposide with cisplatin may be the choice for clinical trials. However, the pharmacokinetic interactions between paclitaxel and etoposide have not been studied yet, and may be important for identifying the optimal schedule of these combinations.

A variety of antitumor agents, including paclitaxel and etoposide, induce apoptosis in human tumor cells, although the exact mechanism of apoptosis in response to these stimuli is unknown. Oncoproteins and tumor suppressor proteins such as bcl-2 and p53 have been shown to modulate the propensity of cells to undergo apoptosis [25, 37]. Bcl-2 overexpression and abnormality of p53 have been shown in a variety of human tumor cells and often correlate with drug resistance and poor prognosis, supporting the in vivo relevance of these proteins. Bcl-2 has been observed to block etoposide-induced apoptosis [25, 37]. Recently, mitotic inhibitors

such as paclitaxel and vinca alkaloids have been shown to induce bcl-2 phosphorylation, which appears to be accompanied by the loss of bcl-2 function, and to induce apoptosis [23]. This suggests that simultaneous exposure to paclitaxel and etoposide or sequential exposure to paclitaxel followed by etoposide may produce synergistic effects against A549, PA1, and MCF7 cells that express bcl-2 protein. However, these schedules did not produce synergistic effects. The loss of bcl-2 function induced by paclitaxel may not necessarily translate into the increased cytotoxicity of etoposide against bcl-2-positive cells.

In conclusion, we demonstrated a schedule-dependent interaction between paclitaxel and etoposide in vitro. Simultaneous exposure to paclitaxel and etoposide showed additive or antagonistic effects, while sequential exposure to paclitaxel followed by etoposide or vice versa showed additive effects, suggesting that the optimal schedule for this combination would be sequential administration, and not simultaneous administration, of the two agents. However, these data are cell line-specific and may therefore be considered as ''artificial'' results of an in vitro study and not representative of the different kinetics and heterogeneity of a human tumor. A synergistic or additive in vitro result does not give an indication of therapeutic index in a live animal or human. Further preclinical and clinical studies should provide further insights and assist in identifying the optimal combination and schedule of paclitaxel and etoposide in clinical use.

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