Table 1
Relationship between proportion of GGO and both pathological findings and recurrence

% GGO	Number of patients	Number of BAC patients	Lymphatic invasion	Vascular invasion	Pleural invasion	Nodal involvement	Recurrence
90–100	14	14	0	0	0	0	0
80–89	8	7	0	0	0	0	0
70–79	4	4	0	0	0	0	0
60–69	3	2	0	0	0	0	0
50–59	2	0	0	0	0	0	0
40–49	10	5	1	0	1	0	0
30–39	7	2	0	1	2	0	1
20–29	8	0	1	1	1	1	0
0–19	34	1	12	9	5	11	7

Four-micrometer sections, including the largest piece cut from the surface of the tumor in each case, were stained with hematoxilin and eosin and elastica van Gieson and examined by means of light microscopy. Intra-tumoral vascular invasion was determined by means of the identification of tumor cells in blood vessels. Lymphatic invasion was also morphologically distinguished from vascular invasion. Pleural invasion was judged as positive if tumor cells invaded across the visceral pleural elastic layer. The tumors were classified into two histologic subtypes according to the classification determined by the World Health Organization (WHO), BAC and other subtypes including acinar, papillary, solid carcinoma with mucin, and adenocarcinoma with mixed subtype [7]. Pathologic stages were classified according to the International System for Staging Lung Cancer criteria [8].

All patients were followed up until death, or the last date of the follow-up (December 31, 2002). The average length of follow-up was 36 months. We investigated the relationship between the proportion of GGO area calculated using our method compared with the pathologic findings and recurrence. The χ^2-test or Fisher's exact test was used to compare several clinical or pathological factors.

3. Results

The distribution of pathologic BAC, nodal status, lymphatic, vascular and pleural invasions, and recurrence by proportion of GGO were shown in Table 1. Among the 90 tumors, 31 (34.4%) were calculated to have a GGO area greater than or equal to 50%. Among the 31 tumors showing a greater GGO proportion ($\geq$ 50%), 27 (87%) tumors were BACs, and no tumors accompanied vessel invasion, pleural invasion, or lymph node metastasis. On the other hand, among the 34 tumors with a GGO area smaller than 20%, 12 (35%) had lymphatic invasion and 11 (32%) accompanied lymph node metastasis. Lymphatic and vascular invasions, or nodal involvement was found more frequently in patients with a smaller proportion of GGO ($<$50%) than patients with a greater proportion of GGO ($\geq$50%) ($P < 0.05$). During the follow-up period, eight patients had tumor recurrences. Of the patients, six were diagnosed as having mediastinal nodal involvement after surgery. There were three local recurrence cases, three distant recurrence cases, and two both local and distant recurrence cases. Seven patients had tumors showing less than 20% of GGO, and one patient had a tumor showing 33% of GGO.

4. Discussion

Detections of nodules showing greater proportion of GGO had increased strikingly since lung cancer screening with low dose CT began [9]. Higashiyama and colleagues investigated the relation between the proportion of BAC component and prognosis. They documented that the greater degree of BAC involvement might reflect the less frequent nodal involvement and good prognosis [10]. We reported the relation between the proportion of GGO and both clinicopathologic characteristics and recurrence in patients with clinical T1N0M0 adenocarcinoma [6]. In this study,

Table 2
Measurement methods of GGO in article

Source	Year	Slice	Method	Parameter	Window setting
Kuriyama et al.	1999	One	Visual	Area	Lung window
Kim et al.	2001	One	Visual	Area	Lung window
Kodama et al.	2001	One	Visual	Area	Lung window
Aoki et al.	2001	One	Measure	Diameter	Lung window
Kondo et al.	2002	One	Visual	Area	Lung /mediastinal window
Matsuguma et al.	2002	All	Visual	Area	Lung window
Takashima et al.	2002	One	Measure	Area	Lung window

the GGO was estimated using visual estimation on all slices in which the tumor appeared. The patients with a higher proportion of GGO area (≥50%) on HRCT had neither lymph node metastasis nor lymphatic invasion and were alive without recurrence. Besides our study, several studies focusing on GGO have been reported to date (Table 2) [11–16]. In many studies including ours, proportions of GGO were semiquantitated by visual estimation. In one study, diameters of nodules and central solid portions were measured instead of area [14]. And in only one study, GGO area was measured using transparent overlay with crossing points of vertical and transverse lines [16]. We think that calculating the area is better than focusing on dimensions because the shape of the central solid portions are often irregular, and sometimes separate as can be seen in our case in Fig. 1.

Standardization for dealing with GGO in selecting candidates for limited resection is urgently needed so that the data from many studies can be compared. Below, we have listed some problems regarding our former published method of measuring GGO. First, visual estimation is somewhat vague and less reproducible. Second, the definition of GGO itself is determined by visual judgment and can result in inter-observer difference. Third, there is a question as to whether the cut-off value of 50% of GGO is or is not the most valuable point in identifying a candidate for limited resection. This is because the cut-off value of 50% was fixed in order to simplify visual judgment. To resolve these problems, we characterized GGO with a CT number, and the proportion of GGO is quantitated more objectively using software. As a result, we obtained almost the same results as our previous study. Furthermore, it has become much clearer that the tumor shows more invasiveness as its proportion of GGO decreases. From our results, the most useful cut-off value for area of GGO may be around 50%, even when using our method. However, future prospective studies are needed to evaluate the effectiveness of limited resection for patients in the early stages of lung cancer based on the objective measurement of GGO. As mentioned above, NIH Image and Scion Image are now freely available. If the images are saved only on the hard-copy film, not as digital data as we have done, you only have to save a few additional images on solid window on hard copy film in addition to the standard lung and mediastinal window images, and transform them into digital data using a scanner. We believe that our methods could be useful and easily available throughout the world.

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Appendix A. Conference discussion

Dr Hyun-Sung Lee (South Korea): Regarding the proportion of GGO, you measured only the area of tumor and GGO, in other words, a two-dimensional evaluation, but I think the proportions of GGO should be

evaluated by the volume, not the area. With the hypothesis that the shape of tumor and GGO is a sphere, the area is proportional to the square of the diameter, but volume is proportional to the squared 3 of the diameter. By its volume or three-dimensional evaluation, the proportion of GGO will lead the different results. I think this is more reliable. What do you think?

Dr Matsuguma: In our previous study we measured the GGO on all slices and in this study we measured on one slice. One slice is two-dimensional and all slices is three-dimensional, so I cannot directly compare these results. We measured the GGO proportion using the software, so we precisely measured GGO. GGO is not equally distributed around the central solid portion, but we measured on both slices of the maximum shadow of the nodule and maximum shadow of the central solid portion. I thought it might almost represent the nature of the GGO tumor.

Dr P. De Leyn (Leuven, Belgium): This entity will gain importance also in West Europe when we will have screening programs. We will see more of these patients than we see now.

When you talk about limited resection, do you mean for nodal dissection, or would you also perform wedge resections for these types of lesions?

Dr Matsuguma: In this study?

Dr De Leyn: Not only in this study, but in your country you see more of these patients and you have a lot of experience. Would you perform wedge resections for these kinds of lesions instead of lobectomy?

Dr Matsuguma: Our limited resection included segmentectomy and wedge resection. In this study there were 10 patients who underwent wedge resection and 7 patients who underwent segmentectomy, that were based on the GGO proportion. Usually we carried out the standard operation for a solid nodule.

Dr F. Rea (Padova, Italy): I don't understand. Do you know the histology before planning your operation? Do you do frozen section? Do you decide, using a frozen section?

Dr Matsuguma: Preoperatively?

Dr Rea: Yes, preoperatively. Do you know preoperatively the diagnosis?

Dr Matsuguma: In many cases we diagnosed preoperatively, but in some cases, such as pure GGO or small nodule, were not diagnosed preoperatively.

Dr Rea: And then you decide with the frozen section!

Dr Matsuguma: Yes.

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ORIGINAL ARTICLE

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Schedule-dependent synergism and antagonism between pemetrexed and paclitaxel in human carcinoma cell lines in vitro

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Abstract Pemetrexed is a novel multitargeted antifolate with significant clinical activity against a variety of tumors. We studied the schedule-dependent cytotoxic effects of pemetrexed in combination with paclitaxel in vitro to improve our understanding of how this combination might be used clinically. Human lung cancer A549 cells, breast cancer MCF7, ovarian cancer PA1, and colon cancer WiDr cells were exposed to both pemetrexed and paclitaxel in vitro. Cell growth inhibition after 5 days was determined and the effects of drug combinations were analyzed by the isobologram method (Steel and Peckham). Simultaneous exposure to pemetrexed and paclitaxel for 24 h produced antagonistic effects in A549 and PA1 cells, additive/antagonistic effects in MCF7 cells, and additive effects in WiDr cells. Pemetrexed for 24 h followed by paclitaxel for 24 h produced synergistic effects in A549 and MCF7 cells and additive effects in PA1 and WiDr cells, while the reverse sequence produced additive effects in all four cell lines. Cell cycle analysis supported these observations. Our findings suggest that the simultaneous administration of pemetrexed and paclitaxel is suboptimal. The optimal schedule of pemetrexed in combination with paclitaxel is the sequential administration of pemetrexed followed by paclitaxel, and this schedule should be assessed in clinical trials for the treatment of solid tumors.

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Keywords Pemetrexed · Paclitaxel · Isobologram · Synergism · Antagonism

Introduction

The development of several new antifolates with distinctive chemical features and target enzymes has provided new opportunities to expand the role of antifolates in cancer chemotherapy. Multitargeted antifolate (MTA, pemetrexed) is a pyrrole-pyrimidine analogue of folate [33] currently in broad clinical evaluation. Pemetrexed is transported into cells mainly through the reduced folate carrier system and metabolized to polyglutamated forms [7] which inhibit thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyl transferase [30, 31], and has antithymidylate and antipurine effects [5]. Preclinical studies of pemetrexed have demonstrated its antitumor activity against a variety of human cancer cells [2, 29].

Phase I studies have shown that the dose-limiting toxicity includes neutropenia and thrombocytopenia, and other toxicities which are manageable, such as mucositis, skin rashes and transient elevations of transaminases [18, 23–25]. Daily and weekly schedules are associated with severe toxicity and 500 mg/m^2 of pemetrexed every 3 weeks was selected as the optimal schedule and dose for the further development of pemetrexed. Patients with a folate-deficient state showed severe toxicity. In preclinical models, folate supplementation reduced toxicity while maintaining antitumor activity. Based on these observations, folate and cobalamin administration before pemetrexed has been routine in recent clinical trials of pemetrexed [9, 26]. Pharmacokinetic studies have shown that pemetrexed undergoes biphasic plasma clearance with a terminal half-life of 1.1–3.1 h, depending on the schedule of administration [23]. The findings from the phase II trial results are encouraging: clear responses were observed in colorectal cancer, pancreatic cancer, lung cancer, breast cancer, mesothelioma, etc. [3, 4, 8, 10, 19–21, 26, 37]. A recent

phase III study has shown that treatment with pemetrexed and cisplatin results in survival times superior to those achieved with cisplatin alone in patients with malignant pleural mesothelioma [39].

Paclitaxel is an established anticancer agent with activity against a variety of solid tumors [1, 6]. Paclitaxel is a mitotic inhibitor that promotes the polymerization and stabilization of tubulin to microtubules [27]. Clinical studies have indicated that neutropenia is the dose-limiting toxicity of paclitaxel [1, 6]. Other toxicities include hypersensitivity reactions, neurotoxicity, mucositis, mild nausea and vomiting, and cardiac injury.

The combination of pemetrexed and paclitaxel may have a major role in the treatment of a variety of solid tumors. The wide range of antitumor activity of pemetrexed and paclitaxel, their different cytotoxic mechanisms and toxic profiles, and the absence of cross-resistance, provide the rationale for using combinations of these agents. Since pemetrexed and paclitaxel are cell cycle-specific agents [17, 38], the disturbances of the cell cycle produced by these agents may influence the cytotoxic effects of each agent, and the drug schedule may play a significant role in the outcome. Therefore, the design of a protocol using them in combination requires careful consideration. As expected, experimental studies for the combination of pemetrexed [22, 30, 36] or paclitaxel [13–15] with other agents have shown schedule-dependent interactions.

The aim of the present study was to elucidate the cytotoxic effects of combinations of pemetrexed and paclitaxel in various schedules on four human carcinoma cell lines. The data obtained were analyzed using the isobologram method of Steel and Peckham [32]. The combination showed schedule-dependent synergism and antagonism.

Materials and methods

Cell lines

Experiments were conducted with the human lung cancer A549, breast cancer MCF7, ovarian cancer PA1, and colon cancer WiDr cell lines. These cells were obtained from the American Type Culture Collection (Rockville, Md.) and maintained in 75-cm^2 plastic tissue culture flasks containing RPMI-1640 medium (Sigma Chemical Co., St Louis, Mo.) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Grand Island Biological Co.) and antibiotics. The cells used were devoid of mycoplasma infection. The doubling times of A549, MCF7, PA1, and WiDr cells under our experimental conditions were in the range 20–24 h.

Drugs

Pemetrexed was kindly provided by Eli Lilly and Company (Indianapolis, Ind.). Paclitaxel was purchased from Bristol-Myers Squibb Japan Co. (Tokyo). The drugs, at a concentration of 1 m*M*, were stored at −20°C and diluted with RPMI-1640 plus 10% FBS prior to use.

Cell growth inhibition using 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 into the individual wells of a 96-well tissue culture plate (Falcon, Oxnard, Calif.). Each plate had one eight-well control column containing medium alone and one eight-well control column containing cells without drug. Eight plates were prepared for each drug combination. The cells were preincubated overnight to allow attachment.

Simultaneous exposure to pemetrexed and paclitaxel

After the overnight incubation for cell attachment, solutions of pemetrexed and paclitaxel (50 µl) at different concentrations were added to the individual wells. The plates were also 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 pemetrexed followed by paclitaxel or the reverse sequence

After overnight incubation, medium containing 10% FBS (50 µl) and solutions (50 µl) of pemetrexed (or paclitaxel) at different concentrations was 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; then fresh medium containing 10% FBS (150 µl) and antibiotics was added, followed by the addition of solutions (50 µl) of paclitaxel (or pemetrexed) 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

Viable cell growth was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously [12]. For all four cell lines examined, we were able to establish a linear relationship between the MTT assay value and the cell number within the range shown.

Isobologram

The dose-response interactions between pemetrexed and paclitaxel for the MCF7, PA1 and WiDr cells were evaluated at the IC_{80} level by the isobologram method (Fig. 1) [32]. The IC_{80} was defined as the concentration of drug that produced 80% cell growth inhibition, i.e., an 80% reduction of absorbance. Since the A549 cells were resistant to pemetrexed and the IC_{80} level was not obtained, the interactions between pemetrexed and paclitaxel were evaluated at the IC_{50} level. We used the isobologram method of Steel and Peckham because this method can cope with any agents with unclear cytotoxic mechanisms and a variety of dose-response curves of anticancer agents [32]. The concept of the isobologram has been described in detail previously [11, 16].

Three isoeffect curves, mode I and mode II, were constructed, based upon the dose-response curves of pemetrexed and paclitaxel (Fig. 1). Mode I and mode II were generated by the assumption regarding overlap and non-overlap damage in combinations, respectively. Thus, when the data points of the drug combination fell within the area surrounded by mode I and/or mode II lines (i.e., within the envelope of additivity), the combination was described as additive. We used this envelope not only to evaluate the simultaneous exposure combinations of pemetrexed and paclitaxel, but also to evaluate the sequential exposure combinations, since the second agent under our experimental conditions could modulate the cytotoxicity of the first agent.

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 (synergistic). 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. The Steel and Peckham isobologram is generally more strict regarding synergism and antagonism than other methods.

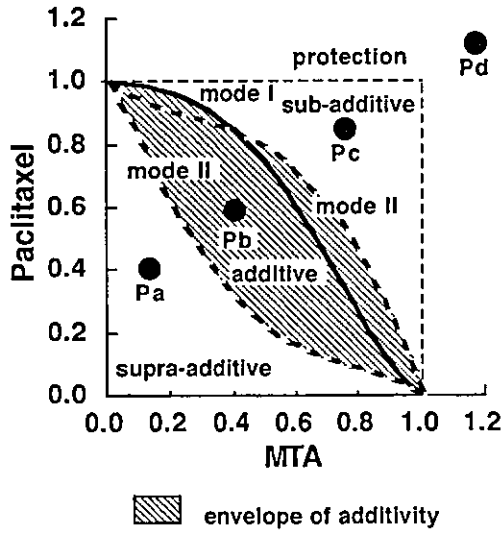

Fig. 1 Schematic representation of an isobologram (Steel and Peckham) [32]. The envelope of additivity, surrounded by mode I (*solid line*) and mode II (*dotted lines*) isobologram lines, was constructed from the dose-response curves of MTA and paclitaxel. The concentrations which produced 80% cell growth inhibition are shown as 1.0 on the ordinate and the abscissa of all isobolograms for MCF7, PA1, and WiDr cells, while the concentrations which produced 50% cell growth inhibition are shown as 1.0 on the ordinate and the abscissa of all isobolograms for A549 cells. Combined data points Pa, Pb, Pc, and Pd show supraadditive, additive, subadditive, and protective effects, respectively

Data analysis

The findings were analyzed as described previously [14]. When the observed data points of the combinations mainly fell in the area of supraadditivity or in the areas of subadditivity and protection, i.e., 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 combinations were considered to have a synergistic or antagonistic effect, respectively. To determine whether the condition of synergism (or antagonism) truly existed, a statistical analysis was performed. The Wilcoxon signed-ranks test was used for comparing the observed data with the predicted minimum (or maximum) values for additive effects, which were closest to the observed data (i.e., the data on the boundary (mode I or mode II lines) between the additive area and supraadditive area (or subadditive and protective areas). Probability (P) values <0.05 were considered significant. Combinations with $P \geq 0.05$ were regarded as indicating additive to synergistic (or additive to antagonistic) effects. All statistical analyses were performed using the Stat View 4.01 software program (Abacus Concepts, Berkeley, Calif.).

Results

The IC_{80} values of pemetrexed for a 24-h exposure against MCF7, PA1, and WiDr cells were 3.3 ± 0.4, 0.15 ± 0.02, and 0.45 ± 0.04 μM, respectively, while those of paclitaxel against MCF7, PA1, and WiDr cells were 5.9 ± 0.4, 2.5 ± 0.06, and 5.8 ± 0.06 nM, respectively. The IC_{50} values of pemetrexed and paclitaxel for a 24-h exposure against A549 cells were 2.5 ± 0.3 μM and 3.4 ± 0.3 nM, respectively.

Figure 2 shows the dose-response curves obtained from simultaneous exposure and sequential exposure to pemetrexed and paclitaxel for the MCF7 cells. The

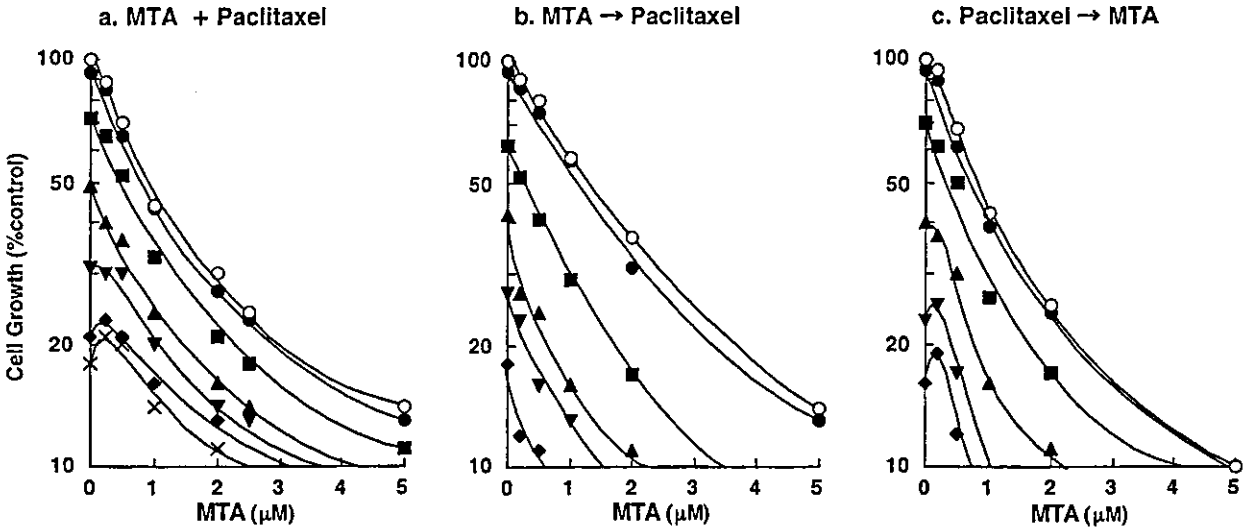

Fig. 2 Schedule dependence of the interaction between MTA and paclitaxel in MCF7 cells. Cells were exposed to (a) these two drugs simultaneously for 24 h, (b) MTA first for 24 h followed by paclitaxel for 24 h, or (c) the reverse sequence. The cell number after 5 days was measured using the MTT assay and was plotted as a percentage of the control (cells not exposed to drugs). The concentrations of MTA are shown on the abscissa. The concentrations of paclitaxel were 0 (*open circles*), 1 (*filled circles*), 2 (*filled squares*), 3 (*filled uptriangles*), 4 (*filled downtriangles*), 6 (*filled diamonds*), and 8 (*crosses*) n*M*, respectively. Data are the mean values for three independent experiments; SE was <20%

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 pemetrexed concentrations are shown on the abscissa. Dose-response curves in which paclitaxel concentrations are shown on the abscissa could be made based on the same data (figure not shown).

Based upon the dose-response curves of pemetrexed alone and paclitaxel alone, three isoeffect curves (mode I and mode II lines) were constructed. Isobolograms at the IC_{80} and IC_{50} levels were generated based upon these dose-response curves for the combinations.

Simultaneous exposure to pemetrexed and paclitaxel for 24 h

Figure 3 shows the isobolograms of the A549, MCF7, PA1, and WiDr cells exposed to both agents simultaneously. For the A549 and PA1 cells, all or most combined data points fell in the areas of subadditivity and protection (Fig. 3a,c). The mean values of the data were larger than those of the predicted maximum data (Table 1). The differences were significant ($P<0.05$ and $P<0.05$), indicating antagonistic effects. For the MCF7 cells, the combined data points fell within the envelope of additivity and in the areas of subadditivity and protection (Fig. 3b; Table 1). The mean value of the data was larger than that of the predicted maximum data. The difference was not significant ($P\geq0.05$), indicating additive/antagonistic effects. For the WiDr cells, the combined data points fell mainly within the envelope of additivity (Fig. 3d). The mean value of the data was larger than that of the predicted minimum data and smaller than that of the predicted maximum data (Table 1), indicating additive effects. A quite similar tendency was observed in the IC_{50} isobologram of the MCF7, PA1, and WiDr cells (not shown).

Sequential exposure to pemetrexed for 24 h followed by paclitaxel for 24 h

Figure 4 shows the isobolograms of the four cell lines exposed first to pemetrexed and then to paclitaxel. For the A549 and MCF7 cells, the combined data points fell in the area of supraadditivity and within the envelope of additivity (Fig. 4a,b). The mean values of the data were smaller than those of the predicted minimum data (Table 1). The differences were significant ($P<0.05$ and $P<0.05$), indicating synergistic effects. For the PA1 cells, the combined data points fell within the envelope of additivity (Fig. 4c), indicating additive effects (Table 1). For the WiDr cells, the combined data points fell within the envelope of additivity and in the area of supraadditivity (Fig. 4d). The mean value of the data was smaller than that of the predicted maximum data and larger than that of the predicted minimum data (Table 1), indicating additive effects. A quite similar tendency was observed in the IC_{50} isobologram of the MCF7, PA1, and WiDr cells (not shown).

Sequential exposure to paclitaxel for 24 h followed by pemetrexed for 24 h

Figure 5 shows the isobolograms of cells exposed first to paclitaxel and then to pemetrexed. For all four cell lines, all or most of the data points fell within the envelope of additivity, indicating additive effects (Table 1). A quite

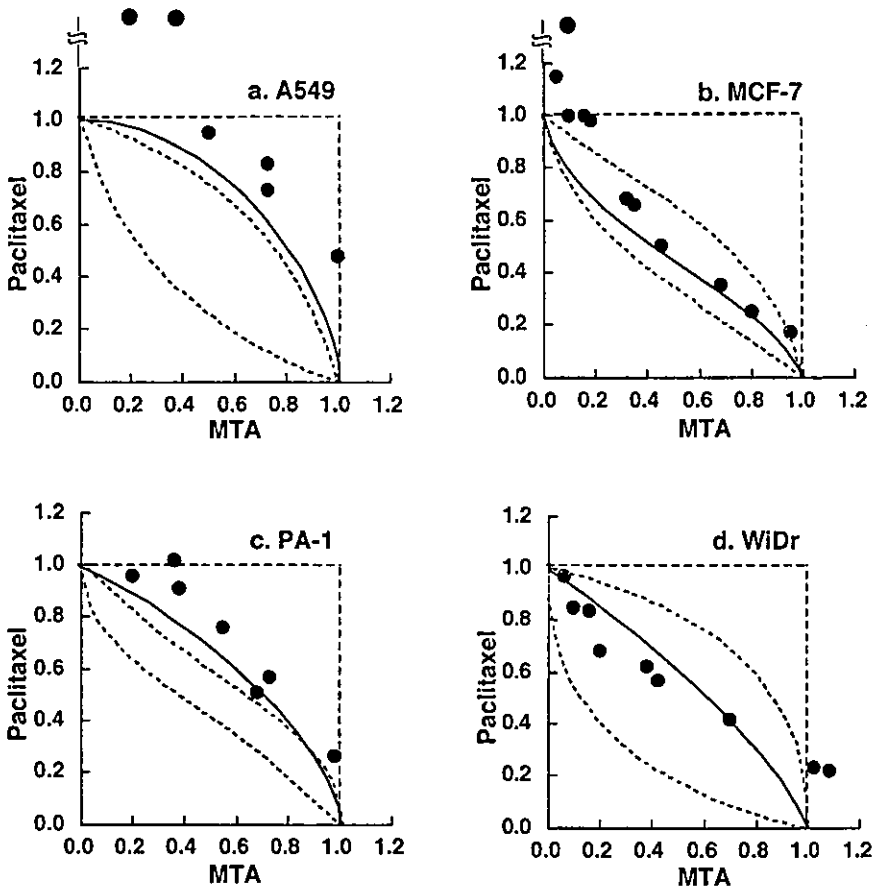

Fig. 3 Isobolograms of simultaneous exposure to MTA and paclitaxel for 24 h in (a) A549, (b) MCF7, (c) PA1, and (d) WiDr cells. For the A549, and PA1 cells, all or most combined data points fell in the areas of subadditivity and protection. For the MCF7 cells, combined data points fell within the envelope of additivity and in the areas of subadditivity and protection. For the WiDr cells, combined data points fell mainly within the envelope of additivity. Data are the mean values for at least three independent experiments; SE was < 30%

similar tendency was observed in the IC_{50} isobologram of the MCF7, PA1, and WiDr cells.

Discussion

We studied the cytotoxic activity of various schedules of pemetrexed in combination with paclitaxel in culture to investigate the optimal schedule of this combination. The analysis of the effects of drug–drug interaction was carried out using the isobologram method of Steel and Peckham [32]. Among the solid tumor cell lines studied, PA1 was most sensitive to pemetrexed, while A549 was most resistant to pemetrexed. The pemetrexed concentrations required for IC_{80} and/or IC_{50} were well within the range that can be attained in human plasma using standard dosing regimens [23].

We demonstrated that cytotoxic interactions between pemetrexed and paclitaxel were schedule-dependent and cell line-dependent. Simultaneous exposure to pemetrexed and paclitaxel showed antagonistic effects in A549 and PA1 cells, additive/antagonistic effects in MCF7

Table 1 Mean values of observed data, predicted minimum, and predicted maximum values of MTA in combination with paclitaxel at IC_{80} for MCF7, PA1 and WiDr cells and at IC_{50} for A549 cells

Schedule	Cell line	*n*	Observed data	Predicted data for an additive effect		Effect
				Minimum	Maximum	
MTA + paclitaxel	A549	6	> 0.92	0.22	0.69	Antagonism ($P < 0.05$)
	MCF7	11	0.61	0.42	0.52	Additive/antagonism
	PA1	7	0.71	0.33	0.60	Antagonism ($P < 0.05$)
	WiDr	9	0.61	0.29	0.78	Additive
MTA → paclitaxel	A549	8	0.31	0.36	0.80	Synergism ($P < 0.05$)
	MCF7	8	0.45	0.60	0.66	Synergism ($P < 0.05$)
	PA1	7	0.41	0.32	0.70	Additive
	WiDr	10	0.34	0.33	0.83	Additive
Paclitaxel → MTA	A549	6	0.78	0.31	0.82	Additive
	MCF7	8	0.58	0.44	0.66	Additive
	PA1	6	0.55	0.44	0.67	Additive
	WiDr	9	0.64	0.25	0.93	Additive

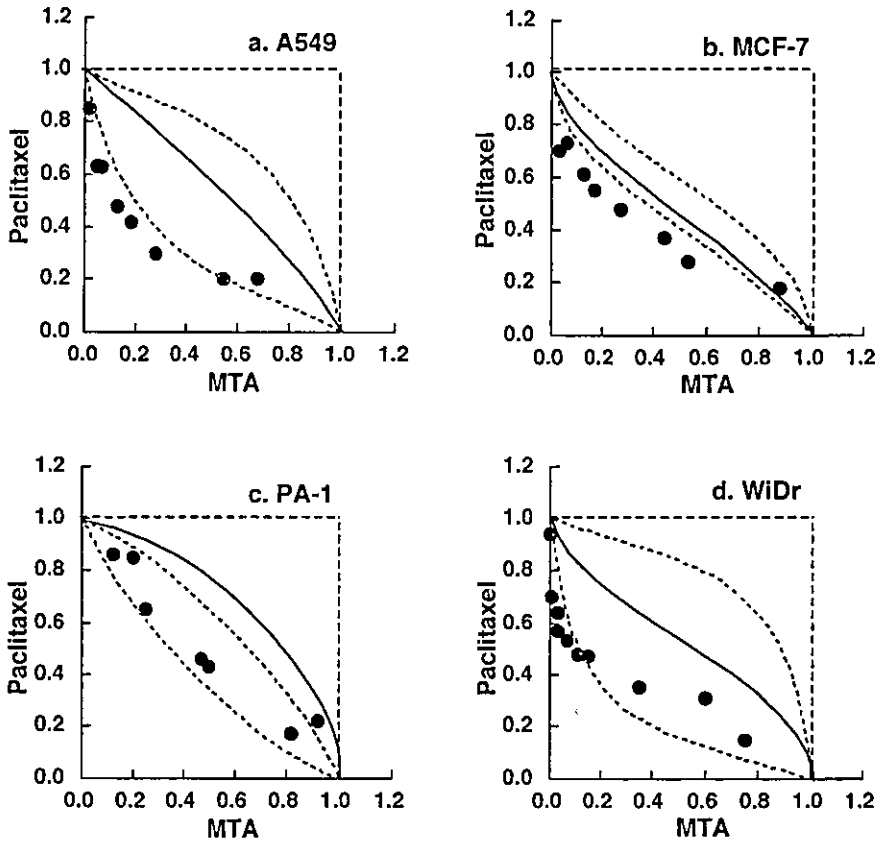

Fig. 4 Isobolograms of sequential exposure to MTA (24 h) followed by paclitaxel (24 h) in (a) A549, (b) MCF7, (c) PA1, and (d) WiDr cells. For the A549 and MCF7 cells, most data points of the combinations fell in the area of supraadditivity. For the PA1 cells, all the data points fell within the envelope of additivity. For the WiDr cells, the data points fell within the envelope of additivity and in the area of supraadditivity. Data are the mean values for at least three independent experiments; SE was <20%

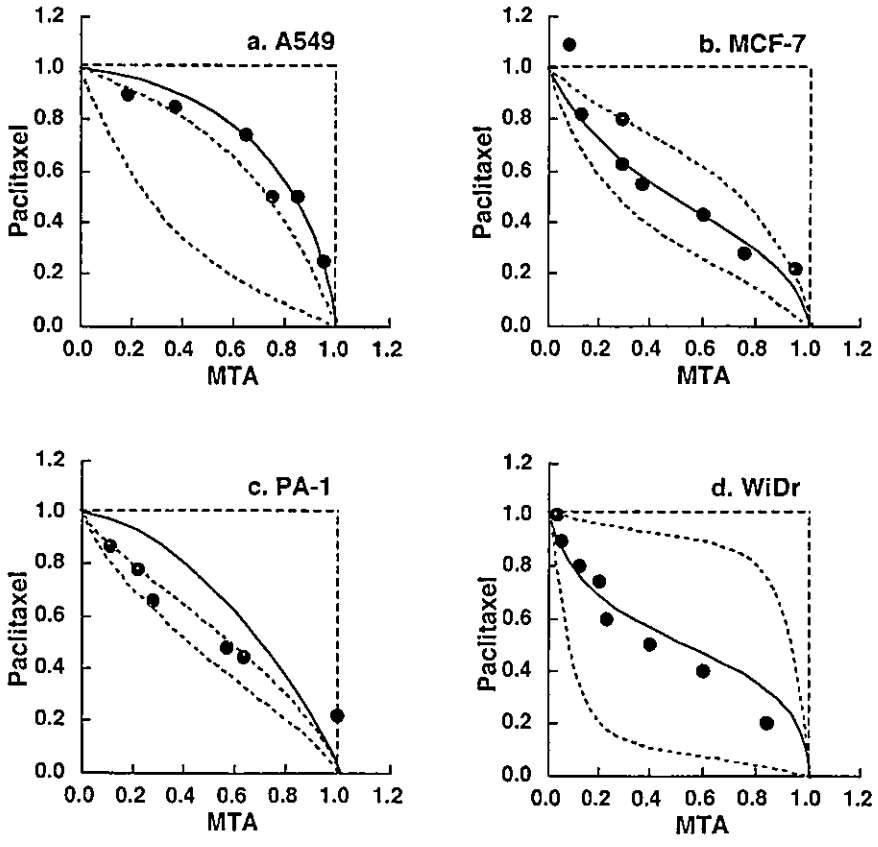

Fig. 5 Isobolograms of sequential exposure to paclitaxel (24 h) followed by MTA (24 h) in (a) A549, (b) MCF7, (c) PA1, and (d) WiDr cells. For all four cells, all or most data points of the combinations fell within the envelope of additivity. Data are the mean values for at least three independent experiments; SE was <25%

cells and additive effects in WiDr cells. Sequential exposure to pemetrexed for 24 h followed by paclitaxel showed synergistic effects in A549 and MCF7 cells and additive effects in PA1 and WiDr cells. However, the combined data points in PA1 and WiDr cells were close to the borderlines between supraadditive and additive areas (Fig. 4), and the observed data were close to the predicted minimum values for an additive effect (Table 1). The combined data points in WiDr cells fell both in the area of supraadditivity and within the envelope of additivity (Fig. 4). Since the isobologram of Steel and Peckham is more strict for synergism and antagonism than other methods for evaluating the effects of drug combinations, simultaneous exposure to pemetrexed and paclitaxel and sequential exposure to pemetrexed followed by paclitaxel would be defined as having antagonistic and synergistic effects, respectively, using other methods.

On the other hand, sequential exposure to paclitaxel followed by pemetrexed showed additive effects in all four cell lines tested. The results of flow cytometric analysis of PA1 cells were consistent with these findings. Enhanced apoptosis was observed only in the pemetrexed–paclitaxel sequence (data not shown).

Our findings suggest that the simultaneous administration of pemetrexed and paclitaxel on the same day is convenient for clinical use but is suboptimal. The sequential administration of pemetrexed followed by paclitaxel may be the optimal schedule for these combinations. For example, administrations of pemetrexed on day 1 and paclitaxel on day 2 would be worthy of clinical investigation. Several in vitro and in vivo studies of combinations of pemetrexed with paclitaxel have been reported [28, 34, 35]. Schultz et al. observed synergistic effects when pemetrexed exposure preceded paclitaxel exposure by 24 h, while the reverse order produced only additive effects in three human cancer cells in vitro [28]. Although the detailed experimental systems are not described in the abstract, our data support their findings.

Teicher et al. studied the combination of pemetrexed and paclitaxel in vivo against EMT-6 murine mammary carcinoma using a tumor cell survival assay [34]. They observed that pemetrexed administered four times over 48 h with paclitaxel administered with the third dose of pemetrexed produced an additive or more than additive tumor response. They further studied the combination of pemetrexed and paclitaxel in human tumor xenografts [35]. Administration of pemetrexed (days 7–11, days 14–18) along with paclitaxel (days 8, 10, 12, and 15) produced greater-than-additive effects on human lung cancer H460 tumor growth delay, while that of pemetrexed (days 7–11) along with paclitaxel (days 7, 9, 11, and 13) produced additive effects on human breast cancer MX-1 tumor growth delay. Since the schedules of administration of pemetrexed with paclitaxel were quite different from ours, comparison seems difficult.

The mechanisms underlying the schedule-dependent synergism and antagonism of the combination of pemetrexed and paclitaxel are unclear. Cell cycle analysis showed that initially exposing cells to pemetrexed leads to synchronization in the S phase (data not shown). Cells in the S phase are sensitive to paclitaxel, in addition to cells in G_2/M phase [17]. This may explain the synergistic effects of sequential exposure to pemetrexed followed by paclitaxel. Simultaneous exposure to pemetrexed and paclitaxel produced antagonistic effects. Pemetrexed has a cytotoxic effect by blocking cells in the S phase [38], while paclitaxel has cytotoxic effects by blocking cells in the G_2/M phase [17, 27]. Thus, one agent might reduce the cytotoxicity of the other agent by preventing cells from entering the specific phase in which the cells are most cytotoxic to the other agent. Interestingly, we have observed similar cytotoxic interactions between methotrexate and paclitaxel [15]. Simultaneous exposure to methotrexate and paclitaxel produces antagonistic effects, while the methotrexate/paclitaxel sequence produces synergistic effects and the reverse sequence produces additive effects. These experimental data suggest that antifolates, which inhibit dihydrofolate reductase, may enhance the cytotoxic action of paclitaxel in sequential administration.

It should be noted that in vitro studies cannot evaluate toxic and pharmacokinetic interactions. Thus, in vivo studies are required to confirm whether the pemetrexed–paclitaxel sequence is optimal or not. In clinical oncology, drug interaction may result in synergism, not only in terms of efficacy but also in terms of toxic side effects. If the toxicities of the drug combinations were compared between the schedules of synergistic and antagonistic interactions at the same doses, the schedules with antagonistic interactions may produce less toxicity than the schedules with synergistic interactions. Our data showed that the drug doses required for IC_{80} or IC_{50} levels with sequential exposure to pemetrexed followed by paclitaxel are less than 70% of the drug doses required for IC_{80} or IC_{50} with simultaneous exposure to the two agents (Figs. 3 and 4). This suggests that the optimal doses for sequential administration of pemetrexed followed by paclitaxel may be lower than those for the simultaneous administration of the two agents. This is important and must be kept in mind for translating in vitro data to clinical applications, since the schedule showing antagonistic effects of the combination may be selected because of less toxicity during the first stage of clinical study.

In conclusion, our findings suggest that the cytotoxic effects of the combination of pemetrexed and paclitaxel are schedule-dependent. The optimal schedule of pemetrexed in combination with paclitaxel is the sequential administration of pemetrexed followed by paclitaxel. Although there are a number of difficulties in the translation of results from in vitro to clinical therapy, this schedule should be assessed in clinical trials for the treatment of solid tumors.

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A Phase II Study of Docetaxel and Infusional Cisplatin in Advanced Non-Small-Cell Lung Cancer

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Key Words
Non-small-cell lung cancer · Chemotherapy · Cisplatin · Docetaxel · Infusion, continuous

Abstract
Background: To evaluate the efficacy and safety of combination chemotherapy of cisplatin (5-day continuous infusion) and docetaxel for the treatment of previously untreated patients with advanced non-small-cell lung cancer (NSCLC). ***Materials and Methods:*** Eligible patients had an ECOG performance status of 0–2 with measurable NSCLC. Patients received continuous infusion cisplatin 20 mg/m^2/day on 5 days and bolus docetaxel 60 mg/m^2/day (day 1; PiD therapy) at a 4-week interval. ***Results:*** Forty-three patients were enrolled. The mean number of cycles administered per patient was 2, and ranged from 1 to 4. The response rate was 49% (95% confidence interval, 33.9–63.8%). The median survival time was 47 weeks and the 1-year survival rate was 47%. The major toxic effects were grade 3 or 4, neutropenia (88%), leukopenia (81%), thrombocytopenia (14%) and anemia (42%). There were no treatment-related deaths. ***Conclusion:*** PiD therapy was a well-tolerated and active regimen for patients with advanced NSCLC. The major toxicity was neutropenia.

Introduction

Unresectable non-small-cell lung cancer (NSCLC) is known to have an extremely poor prognosis, and its standard treatment remains to be established. The most common chemotherapy for NSCLC is a combination treatment consisting of 2 or 3 drugs including cisplatin (CDDP) as a key drug. The combination treatments have response rates of 30–50%, and have been proven to prolong survival time in clinical stages III [1] and IV [2, 3]; however, the response is only limited.

In recent years, new anticancer drugs have been developed and used for the treatment of NSCLC. Docetaxel is a new hemisynthetic anticancer agent originating from its precursor, 10-deacytylbaccatin III, extracted from the needle leaves of the European yew tree, *Taxus baccata* L. Docetaxel affects microtubules, and shows its cytotoxicity by prematurely stabilizing mitotic microtubules. In phase II clinical studies for the treatment of NSCLC carried out in Europe and the USA, docetaxel showed a response rate of about 30% in previously untreated patients with a better survival time [4, 5]. A major side effect of docetaxel is dose-dependent edema that is proportional to bone marrow suppression. Since hypersensitivity is particularly limiting, it is worth noting that docetaxel can be given by intravenous infusion in a short period of time without any pretreatment.

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In the Japan phase I study, dose-limiting toxicity of docetaxel was found to be leukopenia (neutropenia), and its recommended dose was set at 60 mg/m^2 [6]. In the multicenter phase II clinical study for the treatment of NSCLC carried out in Japan, a response rate of 19% was shown in untreated patients with predominant toxicities of leukopenia and neutropenia [7].

Currently, cisplatin is the active agent for treating NSCLC, and combination chemotherapy consisting of 2 or 3 drugs based on CDDP is a major strategy [8]. CDDP can be administered by short-term intravenous infusion, a divided dosage method, continuous administration, and other methods [9, 10]. CDDP cytotoxicity is enhanced by prolonged exposure to low doses of this drug in in vitro studies [11, 12]. Belliveau et al. [13] reported that the area under the concentration-time curve (AUC) achieved for non-protein-bound CDDP was twice as high after 5-day continuous infusion than that observed when an equivalent dose of CDDP was given by short-term bolus infusion. These findings suggest that continuous infusion of CDDP might improve the therapeutic efficacy as compared with that resulting from conventional short-term bolus infusion. However, compared with short-term intravenous infusion, 5-day continuous infusion makes inpatient hospitalization for at least 5 days necessary, and the duration of confinement for the purpose of infusion is lengthy and therefore onerous for the patient. The efficacy and safety of a continuous infusion lasting 5 days (24 h a day) were confirmed in our facility and some other facilities [10, 14–16]. In addition, combination chemotherapy of infusional CDDP with vindesine or CPT-11 was found to have high response rates in treating NSCLC [17, 18].

Cisplatin and docetaxel show nonsynergistic and additive effects in vitro, no cross-resistance and have a relatively nonoverlapping toxicity profile [19]. Therefore, the development of docetaxel in combination with cisplatin is warranted. We conducted a phase II study of docetaxel and infusional cisplatin, in patients with previously untreated advanced NSCLC, and evaluated antitumor activity and the safety of this therapy.

Patients and Methods

Patient Selection

All patients with histologically or cytologically confirmed advanced NSCLC were eligible for this phase II trial. The subjects of this study were patients in clinical stage IV or in stage III with unresectable disease or in whom radiotherapy with curative intent is not possible. Patients with unresectable disease or in whom radiotherapy with curative intent is not possible include those with pleural effusion and dissemination, those with intrapulmonary metastasis within the ipsilateral lobe, those in whom the irradiation field exceeds one half of one lung, those with metastasis to the contralateral hilar lymph nodes, and those with reduced lung function. None of the patients had received prior therapy. Other eligibility criteria included an expected survival of 12 weeks, age ≤75 years, Eastern Cooperative Oncology Group performance score of 0–2, measurable lesions, adequate hematological function (WBC ≥4,000/mm^3, platelet count ≥100,000/mm^3, hemoglobin ≥10 g/dl), renal function (serum creatinine ≤1.5 mg/dl, creatinine clearance ≥60 ml/min), and hepatic function (total serum bilirubin ≤1.5 mg/dl, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase less than twice the normal range). The ethical committee of the Tochigi Cancer Center approved the protocols. Written informed consent was obtained in every case stating that the patient was aware of the investigational nature of this treatment regimen. Pretreatment evaluation included medical history, physical examination, complete blood count, bone marrow examination, serum biochemical analyses, chest roentgenogram, electrocardiogram, and urinalysis. All patients underwent a radionuclide bone scan, and computerized tomography of the brain, thorax and abdomen. Complete blood count, biochemical tests, serum electrolytes, urinalysis, and chest roentgenograms were obtained weekly during this phase II trial. Tests of measurable disease parameters such as computerized tomography were repeated every 4 weeks. Staging was according to the 4th edition of the UICC TNM classification.

Treatment

All patients were admitted to the Tochigi Cancer Center Hospital during this trial. The anticancer drug regimen consisted of a combined administration of docetaxel plus infusional cisplatin. Docetaxel was supplied, in concentrated form, in a sterile vial that contained 80 mg of the drug in 2 ml of polysorbate 80. Docetaxel (Taxotere; Aventis) 60 mg/m^2 was diluted in 250 ml of 5% glucose, and was infused over a 1-hour period on day 1. Three hours after completion of the docetaxel infusion, 20 mg/m^2 of cisplatin was given daily for 5 days by continuous intravenous infusion. One third of the daily dose was administered every 8 h dissolved in 800 ml of physiological saline [14]. The course was repeated every 4 weeks. Antiemetic drugs used were granisetron (3 mg/body/day, bolus infusion for 5 days), metoclopramide (3 mg/kg/day, continuous infusion for 5 days), methylprednisolone (125 mg bolus infusion every 8 h, days 1–5), diphenhydramine (30 mg orally, days 1–7) and alprazolam (1.2 mg orally, days 1–7) [15, 16]. In the first course, no routine premedication was given for hypersensitivity reactions or fluid retention. The reason for this was that the incidence of these events was low at the dose of docetaxel (60 mg/m^2) administered in the present study [7]. However, if hypersensitivity reactions or fluid retention occurred, premedications such as corticosteroids or antiallergic agents were allowed in the subsequent courses. Recombinant human granulocyte colony-stimulating factor was administered when leukopenia/neutropenia of grade 4 occurred.

Patients were treated with at least two cycles of therapy unless disease progression or unacceptable toxicity was encountered or the patients did not wish to continue. Patients who experienced grade 4 leukopenia or neutropenia that lasted for 3 or more days, or who experienced grade 4 thrombocytopenia or reversible grade 2 neurotoxicity or grade 3 liver dysfunction, received reduced doses of

both docetaxel and cisplatin (75% of the previous dose) for the next cycle. Patients who experienced stomatitis of grade 3 or more or renal dysfunction of grade 2 or more received a reduced dose of cisplatin (75% of the previous dose) for the next cycle. If neurotoxicity of grade 3 or more occurred, treatment was stopped. Subsequent courses of chemotherapy were started after day 28 when the leukocyte count was 4,000/mm^3 or more, the neutrophil count was 2,000/mm^3 or more, the platelet count was 100,000/mm^3 or more, serum creatinine was less than the upper limit of the normal range, creatinine clearance was 60 ml/min or more, GOT and GPT were less than twice the upper limit of the normal range, and neurotoxicity was grade 1 or less. If these variables did not return to adequate levels by the first day of the next course of chemotherapy, treatment was withheld until full recovery. If more than 6 weeks passed from the time of the last treatment before these criteria were satisfied, the patient was taken off the study, but still included in the analysis. In the case of stable or progressive disease after two courses of treatment, subsequent therapy was left to the discretion of the physician in charge of the patient.

Assessment of Response to Treatment and Toxicity

The response to treatment was evaluated with WHO criteria. The criteria for response were as follows. Complete response was defined as the complete disappearance of all evidence of tumor for at least 4 weeks. Partial response was defined as a ≥ 50% reduction in the sum of the product of the two greatest perpendicular diameters of all indicator lesions for at least 4 weeks and no appearance of new lesions or progression of any lesion. Progressive disease was defined as a ≥25% increase in the tumor area or the appearance of new lesions. All other circumstances were classified as no change. Toxicity was graded according to the common toxicity criteria (version 2).

Statistical Analyses

The primary end point was the objective response rate. The duration of each response was defined as the number of days from the documentation of the response until tumor progression. Survival curves from registration until death were generated by the method of Kaplan and Meier. We chose a 40% response rate as a desirable target level, and a 20% response rate as undesirable. The study design had the power to detect a response of greater than 90%, with less than 5% error. Therefore, we needed 23 assessable patients in first stage and 20 in second stage, according to the mini-max design of Simon. We decided to stop the study if fewer than 5 patients responded in the first stage.

Results

Patient Characteristics

Forty-three patients were enrolled in this study from July 1997 to June 1999 and received 105 cycles of the regimen. Table 1 shows the patient characteristics. There were 14 women and 29 men with a median age of 61 years (range 34–75). One patient had stage IIIA, 7 patients stage IIIB, and 35 patients stage IV disease. In stage IIIA, 1 patient classified as c-T3N2M0 had lung cancer with a bulky tumor (10 cm), associated with extranodal and N2 involvement. Among the 7 stage IIIB patients, there were three T4 cases in which pleural effusion and pleural dissemination were present, two T4 cases of intrapulmonary metastasis in the ipsilateral lobe, and two T4N3 cases with mediastinal infiltration and supraclavicular fossa lymph node metastasis.

Table 1. Patient characteristics

Patients	43
Sex (M/F)	29/14
Age[1], years	61 (34–75)
Performance status: 0/1/2	9/30/4
Stage: IIIA/IIIB/IV	1/7/35
Histology: Ad/Sq/Other	27/14/2

Ad = Adenocarcinoma; Sq = squamous cell carcinoma.

[1]Value represents median with the range given in parentheses.

Treatments Administered

The mean number of cycles administered per patient was 2, and ranged from 1 to 4. In 99 of 105 cycles (94%), PiD was administered at 4-week intervals. In 5 of 6 cycles, in which cisplatin could not be administered at a 4-week interval, it was given a week later. As for the remaining cycle, it was administered 6 weeks later. The reason for the delay of the administration was the patient's request for 1 cycle and neutropenia in 5 cycles. Dosage was reduced in 7 cycles (7%). Reductions in dosage of docetaxel and cisplatin were made, respectively, in 6 cycles (6%) and 7 cycles (7%). The former reduction was made because 6 cycles showed neutropenia grade 4, and the latter reduction was made because 5 cycles showed neutropenia grade 4, and 1 cycle showed both neutropenia grade 4 and creatinine grade 3, and 1 cycle showed creatinine grade 2.

Response to Treatment and Survival

The response rate was 49% (95% confidence interval, CI, 33.9–63.8%); a complete response was observed in 1 and partial response in 20 patients (table 2). The median duration of the response was 39.2 weeks (range 5–147 weeks). The median survival time was 47 weeks (95% CI, 6–152 weeks) and the 1-year survival rate was 47% (fig. 1). Two patients are still alive.

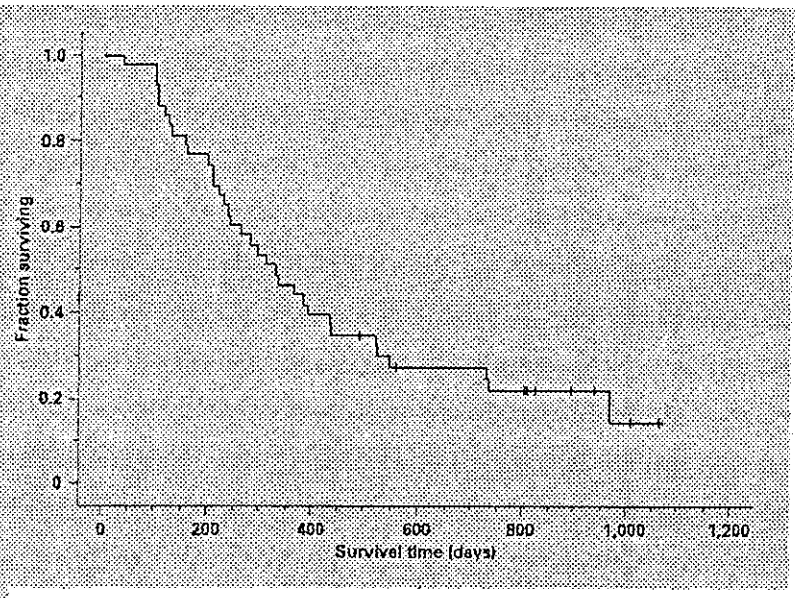

Fig. 1. Kaplan-Meier estimated overall survival curves. Median survival time was 47 weeks; 1-year survival rate was 47%.

Table 2. Chemotherapeutic evaluation (n = 43)

Cycles[1]	2 (1–4)
Response: CR/PR/NC/PD	1/20/20/2
Response rate, %	49
Response duration, weeks	
Average	39.2
Range	5–147
1-year survival rate, %	47

CR = Complete response; PR = partial response; NC = no change; PD = progressive disease.

[1]Value represents average with the range in parentheses.

Table 3. Toxicity (n = 43 patients)

	Maximum toxicity terms of CTC grade					Grade ≥ 3
	0	1	2	3	4	%
Leukopenia	1	1	6	29	6	81
Neutropenia	1	0	4	13	25	88
Anemia	1	6	18	18	–	42
Thrombocytopenia	25	5	7	6	0	14
Creatinine	23	18	1	1	0	2
SGOT/SGPT	30	12	1	0	0	0
Vomiting	5	7	31	0	–	0
Diarrhea	20	16	7	0	0	0
Alopecia	20	22	1	–	–	
Edema	36	6	1	0	–	0
Neuropathy	40	3	0	0	0	0

Figures represent number of patients. CTC = Common toxicity criteria; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase.

Toxicity

Table 3 shows the types and grades of toxicities resulting from the treatment, using the common toxicity criteria. All 43 patients could be evaluated for toxic reactions. The major toxicity was myelosuppression. Leukopenia $<2{,}000/mm^3$ (grade 3 or 4) was observed in 35 patients (81%), of whom 6 patients showed grade 4. Neutropenia $<1{,}000/mm^3$ (grade 3 or 4) was observed in 38 patients (88%), of whom 25 patients showed grade 4. Eight patients developed febrile neutropenia. Thrombocytopenia $<5 \times 10^4/mm^3$ (grade 3 or 4) was observed in 6 patients (14%), and a hemoglobin nadir (grade 3) in 18 patients (42%). There were no episodes of bleeding or fluid overload.

Vomiting grade ≥2 occurred in 31 patients (72%). Diarrhea grade ≥2 was observed in 7 patients (16%). Grade 1 or 2 alopecia and edema were observed in 23 and 7 patients, respectively. In the first cycle, creatinine showed grade ≥2 in 2 patients, resulting in transient rises. In the following cycle, the creatinine level was kept at grade 1 by reducing the dosage of cisplatin. Grade 1 or 2 skin rash was observed in 3 patients. Finally, there were no treatment-related deaths.

Discussion

Cisplatin is one of the key drugs for the treatment of NSCLC. Its high response rate of 40% and safety when it was given alone by continuous infusion over 5 days [14] are confirmed.

Docetaxel is also an active agent to treat NSCLC, and docetaxel of 60 mg/m^2/day (day 1), a recommended dose in Japan, showed a response rate of 19% [7]. Docetaxel has no cross-resistance with cisplatin, and in clinical practice, docetaxel was effective in some patients who were resistant to cisplatin [19]. In addition, additive effects are confirmed between cisplatin and docetaxel, and major side effects of the two drugs are different.

This was a phase II study to determine the usefulness and safety of combination chemotherapy of cisplatin (5-day continuous infusion) and docetaxel for the treatment of advanced NSCLC. The response rate in this study was 49%, which is higher than with docetaxel alone. In comparison with other combination therapies, response rates were 39–42% for cisplatin (bolus) and docetaxel [20, 21], and 58.5% for cisplatin (infusion) and irinotecan with G-CSF. In combination with cisplatin (bolus) and newly developed anticancer agents, the response rates were 44% with paclitaxel [22], 31% with gemcitabine [23], and 26% with vinorelbine [24]. Although these studies differed as regards patients' backgrounds, generally, combination therapies showed better response rates than docetaxel alone.

In our study, side effects predominantly involved hematological toxicity (leukopenia, neutropenia, and anemia). Fever associated with neutropenia was observed in 8 (23%) of 43 patients, and they were treated by administering antibiotics. Hematological toxicities were similar to those in other combination therapies [20, 21]. Nonhematological toxicities were mild, with only 1 patient showing an increased creatinine level of grade 3. The increase was transient, and soon returned to normal. Peripheral edema was observed in only 16%, which was markedly lower than the 24–46% found in other studies [5, 25, 26]. When accumulated doses of docetaxel exceeded 500 mg/m^2, the incidence of edema increased, and at a dose of 85 mg/m^2 or less, eruption was not observed [27]. The dosage was 60 mg/m^2 in our study, and no patients received 500 mg/m^2. There were no side effects concerning hypersensitivity or treatment-related deaths.

We carried out a phase II study of combination treatment of cisplatin (5-day continuous infusion) and docetaxel in 43 patients with NSCLC. The response rate was 49%, and median survival time was 47 weeks. A major side effect was neutropenia. A combination treatment of infusional cisplatin and docetaxel is a tolerable and active regimen for patients with advanced NSCLC. It is to be recommended as a candidate regimen in planning a phase III clinical study in advanced NSCLC, and this regimen will ultimately be evaluated in a phase III clinical study.

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ORIGINAL REPORT

Phase III Randomized Trial of Docetaxel Plus Cisplatin Versus Vindesine Plus Cisplatin in Patients With Stage IV Non–Small-Cell Lung Cancer: The Japanese Taxotere Lung Cancer Study Group

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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ABSTRACT

Purpose
Few randomized trials have demonstrated survival benefit of combination chemotherapy involving new agents plus cisplatin compared with classic combination chemotherapy in advanced non–small-cell lung cancer (NSCLC). The primary aim of this study was to test whether docetaxel plus cisplatin (DC) improves survival compared with vindesine plus cisplatin (VdsC) in patients with previously untreated stage IV NSCLC.

Patients and Methods
Eligible, stage IV, chemotherapy-naive patients (n = 311) were randomly assigned to receive docetaxel 60 mg/m^2 intravenously on day 1 plus cisplatin 80 mg/m^2 intravenously on day 1 of a 3- or 4-week cycle, or vindesine 3 mg/m^2 intravenously on days 1, 8, and 15 plus cisplatin 80 mg/m^2 intravenously on day 1 of a 4-week cycle. Cross-over administration of docetaxel and vindesine was prohibited for both treatment groups.

Results
Overall, 302 patients were eligible for evaluation. The DC arm demonstrated significant improvements compared with the VdsC arm in overall response rates (37% *v* 21%, respectively; $P < .01$) and median survival times (11.3 *v* 9.6 months, respectively; $P = .014$). Two-year survival rates were 24% for the DC arm compared with 12% for the VdsC arm. The physical domain of the Quality of Life for Cancer Patients Treated with Anticancer Drugs measure was significantly better in the DC arm than in the VdsC arm ($P = .020$). Toxicity was predominantly hematologic and was more severe in the VdsC arm.

Conclusion
As first-line treatment for stage IV NSCLC, DC resulted in greater clinical benefit in terms of response rate (with marked improvements in overall and 2-year survival rates) and quality of life than did treatment with VdsC.

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INTRODUCTION

Lung cancer has been a leading cause of cancer death in industrialized countries in the 20th century [1]. Non–small-cell lung cancer (NSCLC) accounts for 75% to 80% of all lung cancer histology. Meta-analyses of randomized trials comparing chemotherapy with supportive care in patients with advanced NSCLC have demonstrated that cisplatin-based combination chemotherapy prolongs survival, whereas some studies showed palliative effects of cancer-related symptoms with chemotherapy [2,3]. Although significant long-term survivors have been observed in the treatment of stage III NSCLC with chemoradiotherapy [4-6], improvements in stage IV disease have been dismal, with only 10% to 15% of stage IV patients surviving 1 year after diagnosis with best supportive care (BSC) alone and 20% to 25% of stage IV patients surviving 1 year

after diagnosis with cisplatin-based chemotherapy [7]. In the 1990s, randomized trials using platinum in combination with new agents (vinorelbine and gemcitabine) have shown 1-year survival rates ranging between 36% and 39% [8,9]. However, many trials have failed to show a significant survival advantage of new compared with older combinations [10-12].

Docetaxel, a new agent, is a semisynthetic taxoid derived from the European yew *Taxus baccata* [13]. It is active against NSCLC and shows survival benefits not only in chemotherapy-naive patients, but also in those patients who have previously received platinum-based chemotherapy [14-21]. Phase II trials of docetaxel and platinum combinations have resulted in median survival rates ranging between 8.4 and 13.9 months, indicating that such combinations are active as first-line therapies [22-25]. Response rates of 30% to 67% for docetaxel with a platinum agent have also been demonstrated. Although docetaxel is usually administered as a 75 mg/m^2 dose, a phase II trial demonstrated that a response rate of 42% with an acceptable toxicity profile [26] could be achieved when 60 mg/m^2 of docetaxel and 80 mg/m^2 of cisplatin were administered to patients with stage IV NSCLC.

We conducted a randomized trial that compared docetaxel plus cisplatin (DC) with vindesine plus cisplatin (VdsC). The primary aim of this study was to compare the overall survival of stage IV NSCLC patients between the two regimens. Secondary end points included the response rate, duration of response, safety, and quality of life (QoL).

PATIENTS AND METHODS

Eligibility Criteria

This multicenter, randomized trial was conducted at 58 institutions in Japan between March 1998 and March 2000. Eligible patients were between the ages of 20 and 75 years, with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2; life expectancy ≥ 3 months; and previously untreated, stage IV, histologically or cytologically proven NSCLC with measurable lesions. Patients with PS of 3 because of pain from bone metastases were admitted to the study. Other eligibility criteria included leukocyte count ≥ 4,000/μL and ≤ 12,000/μL, neutrophil count ≥ 2,000/μL, platelet count ≥ 10^5/μL, hemoglobin ≥ 9.5 g/dL, blood urea nitrogen less than or equal to the upper limit of the institutional normal range (ULN), serum creatinine less than or equal to the ULN, creatinine clearance ≥ 60 mL/min, serum bilirubin less than or equal to the ULN, serum ALT and AST ≤ 2 × ULN, and Pao_2 ≥ 70 mm Hg. Women who were pregnant or lactating were excluded from the study. Other exclusion criteria included patients with active infection, uncontrolled heart disease, interstitial pneumonia or active lung fibrosis, peripheral neuropathy, pleural or pericardial effusion that required drainage, past history of drug hypersensitivity, symptomatic brain metastasis, or active concomitant malignancy.

Patient eligibility was determined by the Patient Registration Center at the Tokyo Cooperative Oncology Group before patient registration. This study was approved by the institutional review boards at each participating center and all patients provided written informed consent.

Treatment Plan

Patients were randomly assigned to one of two treatment arms (Fig 1). In the experimental arm (DC), patients received docetaxel 60 mg/m^2 as a 1-hour intravenous infusion followed by cisplatin 80 mg/m^2 as a 2-hour infusion on day 1. Patients in the control arm (VdsC) received a bolus infusion of vindesine 3 mg/m^2 on days 1, 8, and 15, and cisplatin 80 mg/m^2 as a 2-hour infusion on day 1. Courses of treatment were repeated every 3 to 4 weeks in the DC arm, and once every 4 weeks in the VdsC arm.

Patients received at least two cycles of treatment unless disease progression or unacceptable toxicity was documented. Thereafter, responders or patients without disease progression continued treatment until the appearance of progressive disease or

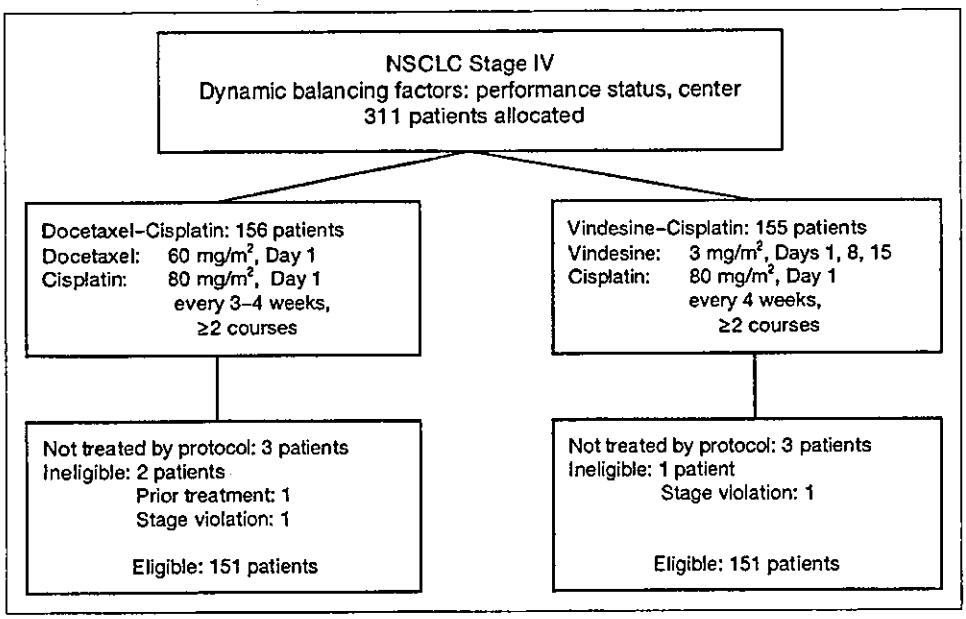

Fig 1. Study design and patient allocation. NSCLC, non–small-cell lung cancer.