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.

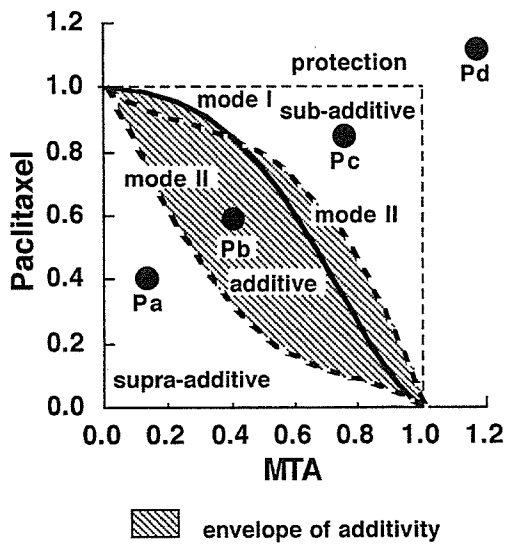

Fig. 1 Schematic representation of an isobologram (Steel and Peckham) [32]. The envelope of additivity, surrounded by mode I (*solid line*) and mode II (*dotted lines*) isobologram lines, was constructed from the dose-response curves of MTA and paclitaxel. The concentrations which produced 80% cell growth 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

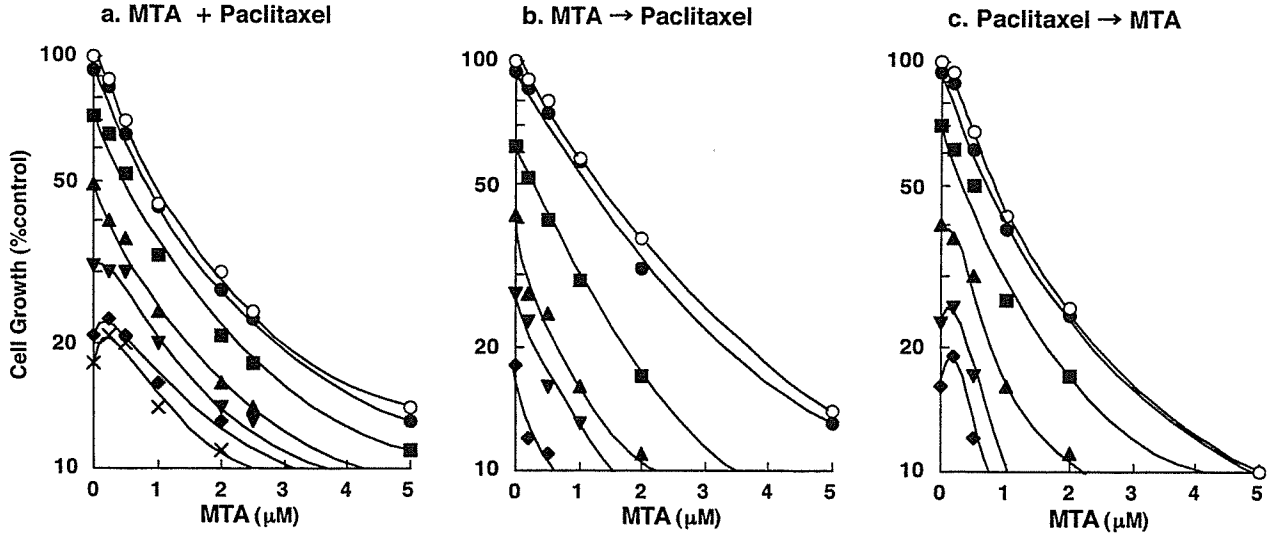

Fig. 2 Schedule dependence of the interaction between MTA and paclitaxel in MCF7 cells. Cells were exposed to (**a**) these two drugs simultaneously for 24 h, (**b**) MTA first for 24 h followed by paclitaxel for 24 h, or (**c**) the reverse sequence. The cell number after 5 days was measured using the 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 ($P \geq 0.05$), 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

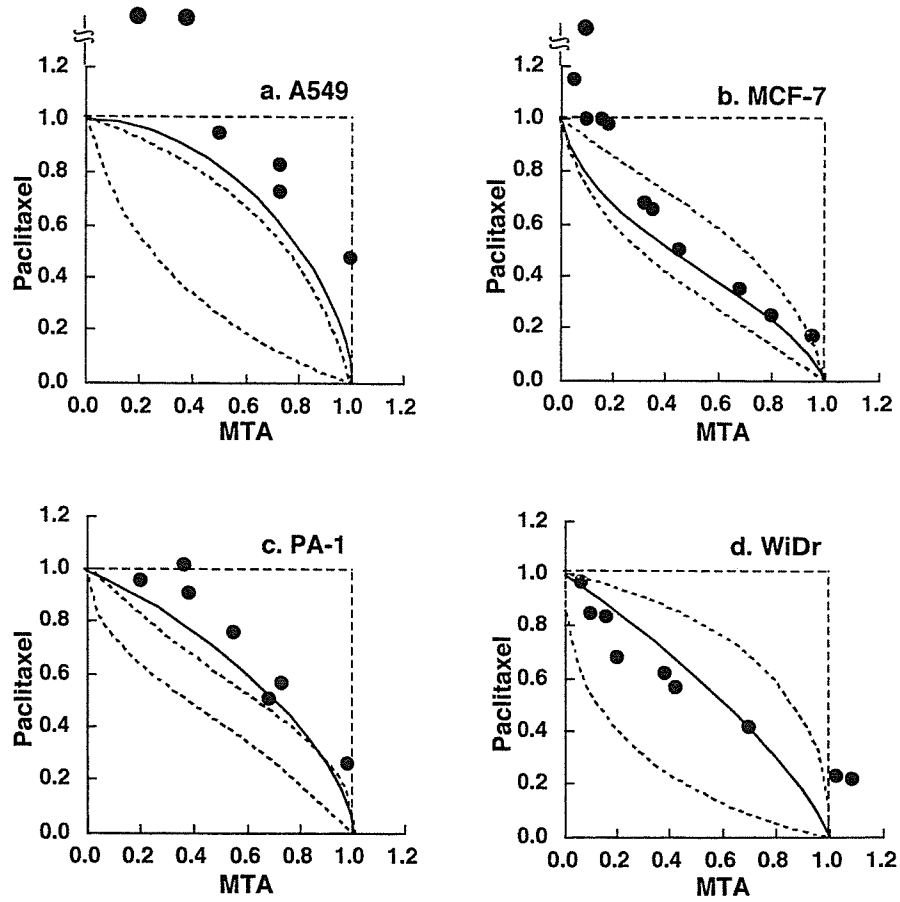

Fig. 3 Isobolograms of simultaneous exposure to MTA and paclitaxel for 24 h in (**a**) A549, (**b**) MCF7, (**c**) PA1, and (**d**) WiDr cells. For the A549, and PA1 cells, all or most combined data points fell in the areas of subadditivity and protection. For the MCF7 cells, combined data points fell 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

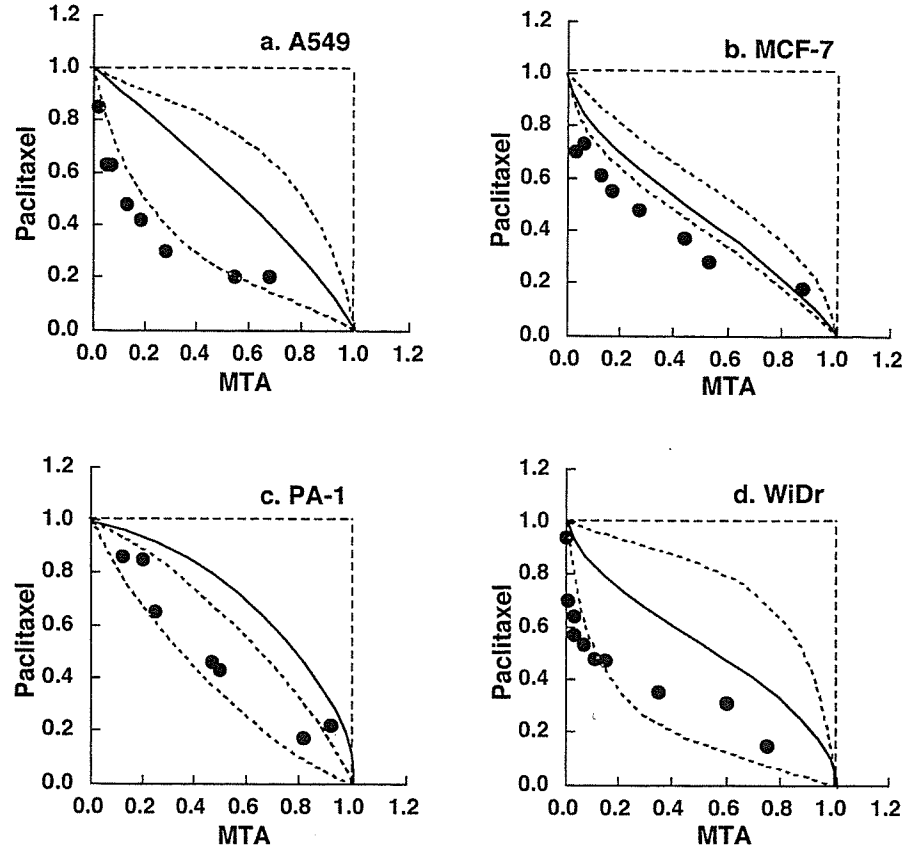

Fig. 4 Isobolograms of sequential exposure to MTA (24 h) followed by paclitaxel (24 h) in (**a**) A549, (**b**) MCF7, (**c**) PA1, and (**d**) WiDr cells. For the A549 and MCF7 cells, most data points of the combinations fell in the area of supraadditivity. For the PA1 cells, all the data points fell 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%

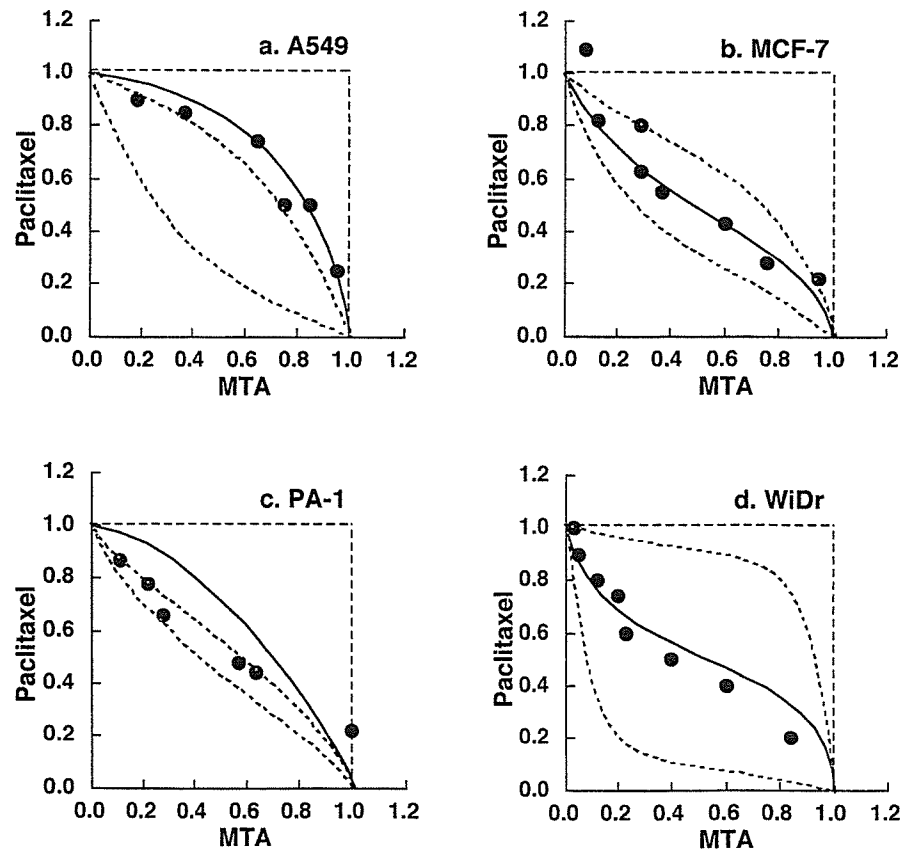

Fig. 5 Isobolograms of sequential exposure to paclitaxel (24 h) followed by MTA (24 h) in (**a**) A549, (**b**) MCF7, (**c**) PA1, and (**d**) WiDr cells. For all four cells, all or most data points of the combinations fell within the envelope of additivity. Data are the mean values for at least three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

Acknowledgments This work was supported in part by a Grant-in-Aid for Cancer Research (11-8) from the Ministry of Health and Welfare and by a Grant-in-Aid for Research on the Second-Term

Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare of Japan.

References

1. Arbuck SG (1995) Paclitaxel: current developmental approaches of the National Cancer Institute. Semin Oncol 22:55–56
2. Britten CD, Izbicka E, Hilsenbeck S, Lawrence R, Davidson K, Cerna C, Gomez L, Rowinsky EK, Weitman S, Van Hoff DD (1999) Activity of the multitargeted antifolate MTA in the human tumor cloning assay. Cancer Chemother Pharmacol 44:105–110
3. Calvert AH, Walling JM (1998) Clinical studies with MTA. Br J Cancer 78 [Suppl 3]:35–40
4. Celio L, Buzzoni R, Longarini R, Marchiano A, Bajetta E (2002) Pemetrexed in gastric cancer: clinical experience and future perspectives. Semin Oncol 29 [Suppl 18]:63–68
5. Chen VJ, Bewley JR, Smith PG, Andis SL, Schultz RM, Iversen PW, Tonkinson JL, Shih C (2000) An assessment of the antithymine and antipurine characteristics of MTA (LY231514) in CCRF-CEM cells. Adv Enzyme Regul 40:143–154
6. Donehower RC, Rowinsky EK (1993) An overview of experience with taxol (paclitaxel) in the USA. Cancer Treat Rev 19 [Suppl C]:63–78
7. Habeck LL, Mendelsohn LG, Shih C, Taylor EC, Colman PD, Gossett LS, Leitner TA, Schultz RM, Andis SL, Moran RG (1995) Substrate specificity of mammalian folypolyglutamate synthetase for 5,10-dideazatetrahydrofolate analogs. Mol Pharmacol 48:326–333
8. Haller DG (2002) Future directions in the treatment of pancreatic cancer. Semin Oncol 29 [Suppl 20]:31–39
9. Hanauske AR, Chen V, Paoletti P, Niyikiza C (2001) Pemetrexed disodium: a novel antifolate clinically active against multiple solid tumors. Oncologist 6:363–373
10. Hochster H (2002) The role of pemetrexed in the treatment of colorectal cancer. Semin Oncol 29 [6 Suppl 18]:54–56
11. Kano Y, Ohnuma T, Okano T, Holland JF (1988) Effects of vincristine in combination with methotrexate and other antitumor agents in human acute lymphoblastic leukemia cells in culture. Cancer Res 48:351–356
12. Kano Y, Sakamoto S, Kasahara T, Akutsu M, Inoue Y, Miura Y (1991) In vitro effects of amsacrine in combination with other anticancer agents. Leuk Res 15:1059–1064
13. Kano Y, Akutsu M, Tsunoda S, Ando J, Matsui J, Suzuki K, Ikeda T, Inoue Y, Adachi K (1996) Schedule-dependent interaction between paclitaxel and 5-fluorouracil in human carcinoma cell lines in vitro. Br J Cancer 74:704–710
14. Kano Y, Akutsu M, Tsunoda S, Suzuki K, Adachi K (1998) In vitro schedule-dependent interaction between paclitaxel and SN-38 (the active metabolite of irinotecan) in human carcinoma cell lines. Cancer Chemother Pharmacol 42:91–98
15. Kano Y, Akutsu M, Tsunoda S, Furuta M, Yazawa Y, Ando J (1998) Schedule-dependent synergism and antagonism between paclitaxel and methotrexate in human carcinoma cell lines. Oncol Res 10:347–354
16. Kano Y, Akutsu M, Tsunoda S, Mano H, Sato Y, Honma Y, Furukawa Y (2001) In vitro cytotoxic effects of a tyrosine kinase inhibitor STI571 in combination with commonly used antileukemic agents. Blood 97:1999–2007
17. Lieu CH, Chang YN, Lai YK (1997) Dual cytotoxic mechanisms of submicromolar taxol on human leukemia HL-60 cells. Biochem Pharmacol 53:1587–1596
18. McDonald AC, Vasey PA, Adams L, Walling J, Woodworth JR, Abrahams T, McCarthy S, Bailey NP, Siddiqui N, Lind MJ, Calvert AH, Twelves CJ, Cassidy J, Kaye SB (1998) A phase I and pharmacokinetic study of LY231514, the multitargeted antifolate. Clin Cancer Res 4:605–610
19. O'Shaughnessy JA, Gennari A, Conte P (2002) Pemetrexed: a promising new treatment for breast cancer. Semin Oncol 29 [2 Suppl 5]:36–41
20. Paz-Ares L, Ciruelos E, Garcia-Carbonero R, Castellano D, Lopez-Martin A, Cortes-Funes H (2002) Pemetrexed in bladder, head and neck, and cervical cancers. Semin Oncol 29 [Suppl 18]:69–75
21. Paz-Ares L, Bezares S, Tabernero JM, Castellanos D, Cortes-Funes H (2003) Review of a promising new agent—pemetrexed disodium. Cancer [Suppl] 97:2056–2063
22. Raymond E, Louvet C, Tournigand C, Coudray AM, Faivre S, De Gramont A, Gespach C (2002) Pemetrexed disodium combined with oxaliplatin, SN38, or 5-fluorouracil, based on the quantitation of drug interactions in human HT29 colon cancer cells. Int J Oncol 21:361–367
23. Rinaldi DA (1999) Overview of phase I trials of multitargeted antifolate (MTA, LY231514). Semin Oncol 26 [Suppl 6]:82–88
24. Rinaldi DA, Burris HA, Dorr FA, Woodworth JR, Kuhn JG, Eckardt JR, Rodriguez G, Corso SW, Fields SM, Langley C, Clark G, Faries D, Lu P, Van Hoff DD (1995) Initial phase I evaluation of the novel thymidylate synthase inhibitor, LY231514, using the modified continual reassessment method for dose escalation. J Clin Oncol 13:2842–2850
25. Rinaldi DA, Kuhn JG, Burris HA, Dorr FA, Rodriguez G, Eckhardt SG, Jones S, Woodworth JR, Baker S (1999) A phase I evaluation of multitargeted antifolate (MTA, LY231514), administered every 21 days, utilizing the modified continual reassessment method for dose escalation. Cancer Chemother Pharmacol 44:372–380
26. Scagliotti GV, Shin DM, Kindler HL, Vasconcelles MJ, Keppler U, Manegold C, Burris H, Gatzemeier U, Blatter J, Symanowski JT, Rusthoven JJ (2003) Phase II study of pemetrexed with and without folic acid and vitamin B_{12} as front-line therapy in malignant pleural mesothelioma. J Clin Oncol 21:1556–1561
27. Schiff PB, Fant J, Horwitz SB (1979) Promotion of microtubule assembly in vitro by taxol. Nature 277:665–667
28. Schultz RM, Dempsey JA, Kraus LA, Schmid SM, Calvete JA, Laws AL (1999) In vitro sequence dependence for the multitargeted antifolate (MTA, LY231514) combined with other anticancer agents. Eur J Cancer 35 [Suppl 4]:S194
29. Shih C, Thornton DE (1998) Preclinical pharmacology studies and the clinical development of a novel multitargeted antifolate, MTA (LY231514). In: Jackman AL (ed) Anticancer drug development guide: antifolate drugs in cancer therapy. Humana, Totowa, p 183
30. Shih C, Chen VJ, Gossett LS, Gates SB, MacKellar WC, Habeck LL, Shackelford KA, Mendelsohn LG, Soose DJ, Patel VF, Andis SL, Bewley JR, Rayl EA, Moroson BA, Beardsley GP, Kohler W, Ratnam M, Schultz RM (1997) LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. Cancer Res 57:1116–1123
31. Shih C, Habeck LL, Mendelsohn LG, Chen VJ, Schultz RM (1998) Multiple folate enzyme inhibition: mechanism of a novel pyrrolopyrimidine-based antifolate LY231514 (MTA). Adv Enzyme Regul 38:135–152
32. Steel GG, Peckham MJ (1979) Exploitable mechanisms in combined radiotherapy–chemotherapy: the concept of additivity. Int J Radiat Oncol Biol Phys 5:85–91
33. Taylor EC, Kuhnt D, Shih C, Rinzel SM, Grindey GB, Barredo J, Jannatipour M, Moran RG (1992) A dideazatetrahydrofolate analogue lacking a chiral center at C-6, *N*-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid, is an inhibitor of thymidylate synthase. J Med Chem 35:4450–4454
34. Teicher BA, Alvarez E, Liu P, Lu K, Menon K, Dempsey J, Schultz RM (1999) MTA (LY231514) in combination treatment regimens using human tumor xenografts and the EMT-6 murine mammary carcinoma. Semin Oncol 28:55–62
35. Teicher BA, Chen V, Shih C, Menon K, Forler PA, Phares VG, Amsrud T (2000) Treatment regimens including the multitargeted antifolate LY231514 in human tumor xenografts. Clin Cancer Res 6:1016–1023

36. Tesei A, Ricotti L, De Paola F, Amadori D, Frassineti GL, Zoli W (2002) In vitro schedule-dependent interactions between the multitargeted antifolate LY231514 and gemcitabine in human colon adenocarcinoma cell lines. Clin Cancer Res 8:233–239
37. Tomek S, Emri S, Krejcy K, Manegold C (2003) Chemotherapy for malignant pleural mesothelioma: past results and recent developments. Br J Cancer 88:167–174
38. Tonkinson JL, Marder P, Andis SL, Schultz RM, Gossett LS, Shih C, Mendelsohn LG (1997) Cell cycle effects of antifolate antimetabolites: implications for cytotoxicity and cytostasis. Cancer Chemother Pharmacol 39:521–531
39. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, Gatzemeier U, Boyer M, Emri S, Manegold C, Niyikiza C, Paoletti P (2003) Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 21:2636–2644

Lung Cancer (2006) 53, 197–203

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A combination chemotherapy of carboplatin and irinotecan with granulocyte colony-stimulating factor (G-CSF) support in elderly patients with small cell lung cancer☆

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Received 23 January 2006; received in revised form 23 April 2006; accepted 9 May 2006

KEYWORDS
Small cell lung cancer;
Elderly;
Chemotherapy;
Carboplatin;
Irinotecan

Summary

Background: We have previously reported that carboplatin plus etoposide is an effective and relatively non-toxic regimen in elderly patients with small cell lung cancer (SCLC). Recently, the Japan Clinical Oncology Group reported that irinotecan plus cisplatin was more effective than etoposide plus cisplatin in the treatment of non-elderly patients with extensive disease (ED)-SCLC. Therefore, we conducted a prospective feasibility study designed specifically to evaluate the efficacy of carboplatin (day 1) and irinotecan (days 1, 8, 15) with granulocyte colony-stimulating factor (G-CSF) support in elderly SCLC patients.

Methods: Three carboplatin AUC and irinotecan dose levels were used: 4 mg/ml × min and 50 mg/m^2, respectively (level 1); 5 mg/ml × min and 50 mg/m^2, respectively (level 2), and 5 mg/ml × min and 60 mg/m^2, respectively (level 3). Although a phase I trial using this drug combination against non-SCLC performed at our institution found that the recommended dose was level 3, as the current trial included only elderly patients, the starting dose used was level 2. However, if a patient had history of prior chemotherapy, performance status (PS) of 2, or was aged 75 years or more, the dose administered was reduced by 1 level. If a patient had a PS of 0, the dose was increased by 1 level. Cycles were repeated every 4 weeks, and patients aged 70 years or more with a PS of 0–2 were eligible.

Results: Eighteen patients were enrolled, of which nine were given the level 1 dose, seven the level 2 dose, and two the level 3 dose. The patient group had a median age of 75 years, 8 patients had limited disease (LD) versus 10 with ED, 9 had received previous treatment for SCLC versus 9 previously untreated, and 13 had a PS of 0–1 versus 5 with a PS of 2. Seventeen (94%) patients

☆ Presented in part at the 40th Annual Meeting of the American Society of Clinical Oncology, New Orleans, Louisiana, June 5–8, 2004.

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0169-5002/$ – see front matter
doi:10.1016/j.lungcan.2006.05.004

received two or more cycles of chemotherapy, and the median actual delivery of irinotecan was 84% of the projected dose. Grade 3/4 neutropenia, anemia, and diarrhea occurred in 50%, 33% and 6% of patients, respectively. Other toxicities were mild and no treatment-related deaths occurred. The response rate was 89%, with two complete responses and 14 partial responses. The median survival time was 13.3 months and the 1-year survival rate was 62%.
Conclusions: The combination of carboplatin and irinotecan with G-CSF support was an effective and non-toxic regimen in elderly SCLC patients and should be further evaluated in phase III trials.

1. Introduction

Approximately half of the patients with small cell lung cancer (SCLC) are older than 70 years, and this proportion of elderly SCLC patients is expected to increase in Japan [1–3]. Commonly used combination chemotherapy regimens for non-elderly SCLC include: cyclophosphamide, doxorubicin, and vincristine (CAV); cisplatin and etoposide (PE); alternating PE/CAV; and irinotecan plus cisplatin (IP) [4–6]. However, since many studies arbitrarily exclude elderly patients from clinical trials, no standard chemotherapeutic regimen has yet been established for elderly SCLC patients.

The Japan Clinical Oncology Group (JCOG) concluded that carboplatin plus etoposide (CE) represented an effective regimen with low toxicity in elderly SCLC patients in a phase II trial [7], and showed that IP was more effective than PE in the treatment of non-elderly patients with extensive disease (ED)-SCLC in a phase III trial [6]. As few clinical trials have evaluated the role of irinotecan in elderly patients with SCLC, we decided to conduct a prospective feasibility study designed to evaluate the efficacy of the carboplatin plus irinotecan (CI) regimen in elderly SCLC patients.

2. Patients and methods

2.1. Patient selection

Eligibility criteria were previously treated or untreated patients with histologically or cytologically confirmed SCLC, ≥70 years in age, and with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2. Additional criteria were the presence of limited disease (LD) or ED (all stages of SCLC were eligible), presence of evaluable or measurable disease, expected survival ≥2 months, adequate organ function [leukocyte count ≥4000/mm^3, platelet count ≥100,000/mm^3, hemoglobin level ≥9.0 g/dl, AST/ALT ≤2 × upper limit of normal range, total bilirubin ≤1.5 mg/dl, creatinine ≤1.5 mg/dl, creatinine clearance (Ccr) ≥50 ml/min, and PaO_2 ≥60 mmHg], absence of pericardial or pleural effusions requiring drainage, absence of active concomitant malignancy, no senile dementia, and written informed consent. ED was defined as presence of distant metastases, contralateral hilar-node metastases, or pleural effusion. Exclusion criteria included brain metastases or superior vena cava (SVC) syndrome that required radiotherapy, and serious medical or psychiatric illness. Staging procedures included chest X-ray, computed tomography (CT) scan of the chest, CT scan or magnetic resonance imaging (MRI) of the brain, CT scan or ultrasound of the abdomen, and isotope bone scanning.

2.2. Treatment protocol

Treatment consisted of carboplatin administered intravenously on day 1 plus irinotecan administered intravenously on days 1, 8, and 15. Granulocyte colony-stimulating factor (G-CSF) at 50 μg/m^2 or 2 μg/kg was administered daily except on days 1, 8, 15, until leukocyte counts exceeded 10,000/mm^3, at which point the G-CSF was discontinued. If leukocyte counts decreased to less than 3000/mm^3, G-CSF treatment was restarted. Cycles were repeated every 4 weeks for up to four courses. This trial used three carboplatin area under the curve (AUC) and irinotecan dose levels of 4 mg/ml × min carboplatin and 50 mg/m^2 irinotecan (level 1), 5 mg/ml × min carboplatin and 50 mg/m^2 irinotecan (level 2), and 5 mg/ml × min carboplatin and 60 mg/m^2 irinotecan (level 3). Based on a phase I trial of combined carboplatin and irinotecan for non-SCLC performed at our institution, level 3 was determined to be the recommended dose [8]. However, as the current trial included only elderly patients, the starting dose was reduced to level 2. If a patient had history of prior chemotherapy, performance status (PS) of 2, or was 75 or more years old, the dose administered was reduced by 1 level. If a patient had a PS of 0, the dose was increased by one level. For example, if a patient had a PS of 0 and was 78-years old, the patient received level 2 dose. If a patient had a PS of 2 and was 73-years old, the patient received level 1 dose. The 24 h Ccr was substituted for glomerular filtration rate (GFR) in Calvert's formula [9]. Antiemetic prophylaxis with 5-HT_3 antagonists plus dexamethasone was routinely used. In cases of irinotecan-induced diarrhea, high dose loperamide treatment was given as described in Abigerges et al. [10]. Irinotecan was withdrawn if leukocyte counts were less than 3000/mm^3, platelet counts less than 75,000/mm^3, or if diarrhea of grade 1 or more occurred on days 8 and 15. Subsequent courses of chemotherapy were initiated when leukocyte counts reached 4000/mm^3 and platelet counts 100,000/mm^3 after day 28 and for 2 or more days after the discontinuation of G-CSF. If the above criteria were not satisfied by the first day of the next course, treatment was withheld until full recovery. If more than 6 weeks passed from the first day of the last course, the patient was taken out of the study. Dose modifications were made for both carboplatin and irinotecan based on toxicity. Patients that experienced grade 4 leukopenia or neutropenia, grade 2 diarrhea, or neutropenic fever received a 25% reduction in irinotecan dose for the next course. Patients that experienced grade 3 or 4 thrombocytopenia received a 20% reduction in target carboplatin AUC for the next course. If the same toxicity occurred following dose reduction, the patient was taken out of the study. If grade 3 or 4 non-hematologic toxici-

ties, except for nausea/vomiting and hyposodium, occurred, the patient was taken out of the study even if the toxicities improved thereafter. Patients with LD received thoracic irradiation after chemotherapy. Palliative radiotherapy of less than 20 Gy total dose was allowable to control persistent pain associated with bone metastasis during the study period. After the completion of four courses, responders did not receive further chemotherapy unless progressive disease (PD) developed. Post-protocol treatments were left at the discretion of the physician. Prophylactic cranial irradiation (PCI) was an option for patients that achieved a complete response (CR).

2.3. Evaluation

Tumor responses were evaluated according to World Health Organization criteria [11]. A CR was defined as the complete disappearance of tumor for at least 4 weeks. A partial response (PR) was defined as a ≥50% reduction in the sum of the products of the two greatest perpendicular diameters of all indicator lesions or a reduction of more than 50% in assessable disease for at least 4 weeks, with no appearance of new lesions or progression of any existing lesions. PD was defined as a ≥25% increase in tumor area or the appearance of new lesions. All other outcomes were classified as no change (NC). At the time of study initiation in March 1998, response evaluation criteria in solid tumors (RECIST) [12] was not yet available, such that toxicities were evaluated according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC).

2.4. Study design and statistics

This trial was designed as a prospective non-phase I study and the main objective is to see feasibility and efficacy. The study protocol was approved by the institutional review board at our institution prior to the initiation of the study. Study objectives were to detect and quantify the clinical toxicities of the carboplatin and irinotecan combination and to assess its therapeutic efficacy in elderly patients with SCLC. Because this feasibility study included a heterogeneous patient population, (e.g. in terms of presence of prior chemotherapy and disease stage), the study was not designed as a phase I or II study. Therefore, sample size calculations based on Simon's minimax design were not applied to this study. Analysis of the trial was based on the intention-to-treat principle. Overall survival, determined from the time of registration to death or the last follow-up evaluation, was calculated using the Kaplan and Meier method.

3. Results

3.1. Patient characteristics

Between March 1998 and December 2003, 18 patients were registered for the study, and all received chemotherapy. Patient characteristics are listed in Table 1. Patients consisted of 4 women and 14 men, with a median age of 75 years (range, 70–85 years) and a median 24 h Ccr of 74 ml/min (range, 28–134 ml/min). Thirteen patients (72%) had an ECOG PS of 0 or 1. Eight patients had LD and 10 had ED. Nine patients had a history of prior chemotherapy (five with sensitive relapses, four with refractory relapses) and nine were chemo-naïve. Of the previously treated patients, five had received one regimen of CE and two had received one regimen of PE. One patient had received two regimens consisting of CE and CODE (cisplatin + oncovin + doxorubicin + etoposide), and one patient had received three regimens consisting of CE, CODE and IP. The numbers of patients that started at dose levels 1, 2 and 3 were nine, seven and two, respectively (Table 2).

Table 1 Patient characteristics

No. of patients	18
Male/female	14/4
Median age, years (range)	75 (70–85)
Stage: LD/ED	8/10
PS (ECOG): 0/1/2	4/9/5
Prior chemotherapy: present/absent	9/9
Sensitive/refractory cases	5/4
Median 24 h Ccr, ml/min (range)	74 (28–134)

LD, limited disease; ED, extensive disease; PS, performance status; ECOG, Eastern Cooperative Oncology Group; Ccr, creatinine clearance.

Table 2 Dose level

Level	No. of patients	AUC of carboplatin (mg/ml × min)	Dose of irinotecan (mg/m^2)
1	9	4	50
2	7	5	50
3	2	5	60

AUC, area under the curve.

3.2. Treatment delivery

Nine patients (50%) received four courses of treatment, two (11%) received three courses, six (33%) received two courses, and one (6%) received one course. The reasons for termination of treatment included completion of two or more courses of chemotherapy (16 patients, 89%), and NC (two patients, 11%). One patient experienced grade 3 diarrhea after receiving a single course of chemotherapy and was taken off the study. No treatment-related deaths (TRDs) occurred. Course intervals and dose reductions are listed in Table 3. The median interval of each round of chemotherapy was 28–29 days. Only four patients received a reduced dose

Table 3 Course interval and dose reduction

Interval of each chemotherapy course	No. of patients	Median days (range)	No. of patients with dose reduction
1–2	17	28 (21–35)	4[a]
2–3	10	29 (25–36)	0
3–4	9	28 (27–35)	0

[a] Thrombocytopenia, two patients; neutropenia, one patient; both, one patient.

Table 4 Hematologic toxicity (worst of any course)

Level	No. of patients	Leukopenia			Neutropenia			Anemia			Thrombocytopenia		
		Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4
1	9	5	0	0	2	4	0	5	3	0	2	0	0
2	7	2	3	0	1	3	1	2	3	0	2	1	2
3	2	1	1	0	1	1	0	2	0	0	0	0	0

Table 5 Non-hematologic toxicity (worst of any course)

Level	No. of patients	Nausea/vomiting				Diarrhea				Infection			Pulmonary		Liver		Renal	
		Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade ≥3	Grade 2	Grade ≥3	Grade 1	Grade ≥2	Grade 2	Grade ≥3
1	9	5	0	1	0	3	3	1	0	1	1	0	0	0	1	0	0	0
2	7	7	0	0	0	3	2	0	0	0	0	0	0	0	1	0	0	0
3	2	2	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0

Table 6 Therapeutic response

Level	No. of patients	Previously treated patients (refractory)	Response			
			CR	PR	NC	PD
1	9	6 (3)	1	6	2	0
2	7	3 (1)	1	6	0	0
3	2	0	0	2	0	0

Overall response rate (ORR) = 16/18 (89%); RR for previously untreated patients = 9/9 (100%); RR for previously treated patients = 7/9 (78%). CR, complete response; PR, partial response; NC, no change; PD, progressive disease.

in the second course due to toxicities experienced during the first course. The reasons for dose reduction were thrombocytopenia in two patients, neutropenia in one patient and both thrombocytopenia and neutropenia in one patient. However, no patients experienced further toxicities after dose reduction. Median percentage of irinotecan dose intensity (mg/m^2/week), expressed as the actual delivered dose as a percentage of the projected dose, was 84% (range: 48—100%). Of the 162 projected irinotecan infusions, 18 dose omissions occurred during the study period due to leukopenia in five cases, thrombocytopenia in four cases, diarrhea in eight cases and patient refusal in one case. Therefore, the percentage of actual irinotecan infusions, based on actually delivered infusions as a percentage of projected infusions, was 89% (144/162).

3.3. Toxicity

Hematologic and non-hematologic toxicities are listed in Tables 4 and 5. Grade 3 or 4 neutropenia, anemia, and thrombocytopenia occurred in 50%, 33%, and 17% of patients, respectively. However, neither grade 4 leukopenia nor anemia occurred at all three dose levels. Non-hematologic toxicities were generally mild, and grade 3 diarrhea and grade 3 nausea/vomiting occurred in only one patient each. Other non-hematologic toxicities were also mild, and no grade 3 or 4 toxicities except for gastrointestinal toxicities occurred at all three dose levels.

3.4. Response and survival

Chemotherapeutic responses are listed in Table 6. Of the 18 patients, two showed CRs and 14 PRs, giving a response rate of 89% (16/18). For the nine chemo-naïve patients, the response rate was 100% (9/9). In contrast, of the nine previously-treated patients, seven responded to treatment, giving a response rate of 78% (7/9). Of the four patients with refractory relapses, two responded. The median survival time (MST) and 1-year survival rate for all 18 patients in the study was 13.3 months and 62%, respectively (Fig. 1).

4. Discussion

Until recently, there was no standard chemotherapeutic regimen for elderly SCLC patients. However, four comparative studies, including two phase III [13,14] and two randomized phase II [15,16] trials, have shown that suboptimal chemotherapies, such as oral etoposide monotherapy or attenuated doses of combination chemotherapy, may lead to reduced survival in elderly or poor-risk SCLC patients when compared with standard doses of combination chemotherapies.

To our knowledge, this is the first study to evaluate the CI regimen in elderly patients with SCLC. The response rate of the CI regimen was 89%, with an MST of 13.3 months. These were very promising results, especially as this study included only elderly SCLC patients and half of the study group had already received some form of chemotherapy, although this study included both ED and LD patients as the same population. Observed instances of toxicity tended to be mild and no TRDs occurred. Although a near full-dose combination chemotherapy was administered to the elderly SCLC patients in our study, only half of the patients experienced grade 3/4 neutropenia. Furthermore, the irinotecan dose intensity of 84% was relatively high. It is possible that the acceptable toxicities and dose intensity were largely attributable to the prophylactic use of G-CSF and the high-dose loperamide therapy against irinotecan-induced diarrhea. On the other hand, other phase I studies, which also included patients over the age of 70, demonstrated that carboplatin AUC 5 and irinotecan 50 mg/m^2 can be safely administered without G-CSF prophylaxis [17—19]. However, these studies were not specifically designed to the elderly population and the median age of these studies were clearly younger than that of our trial.

Several retrospective analyses [20—22] and a prospective study [23] have shown that standard-dose chemotherapy without G-CSF support can lead to an increased risk of early death and sepsis in older populations. Moreover, American

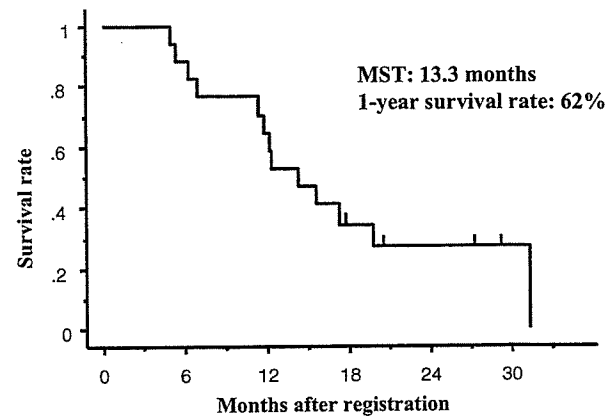

Fig. 1 Overall survival curve.

Society of Clinical Oncology (ASCO) guidelines recommend the use of prophylactic G-CSF in patients at higher risk of chemotherapy-induced infections, including patients with a poor PS or comorbid illness [24]. Therefore, we suggest that the prophylactic use of G-CSF in this study was justified as the CI regimen used was near to the full-dose regimen even though only elderly patients with SCLC were studied.

As our study consisted of a heterogeneous patient population, including patients that had been previously treated, or over 75 years of age, three dose levels were used according to individual patient characteristics. Furthermore, stage was also different among the patients. Therefore, the limitation of this study was that it was neither considered phase I nor II study and was not designed based on the proper statistical methodology. However, at the time of study proposal, no prospective trial using carboplatin plus irinotecan regimen for elderly patients with SCLC was reported. Furthermore, we did not know whether this combination was feasible and effective for elderly SCLC patients. Therefore, dose levels were selected by patient characteristics and this study was designed as a prospective study to evaluate feasibility and efficacy for the elderly SCLC patients. For this reason, it may be difficult to mention on the efficacy of this treatment because of wide patient selection and uncommon study design. In terms of future trials using the CI regimen, level 1 or 2 appeared to be the appropriate dose level for previously untreated elderly patients with adequate organ function because majority of the patients were registered in level 1 and 2. However, phase I/II study using the CI regimen, which is based on the proper statistical method, is warranted for evaluating toxicity and efficacy in the chemo-naïve elderly SCLC patients with specific stage.

Recently, we reported a phase III trial that compared the CE regimen to a split doses of PE (SPE) regimen in elderly or poor-risk patients with ED-SCLC (JCOG 9702) [25]. Although the CE regimen led to pronounced but manageable thrombocytppenia, other toxicities, palliation scores, response rate, and overall survival rate were very similar between the two treatments. However, the CE regimen did not require hydration and could be given in an outpatient setting. Based on the results of this phase III study, many JCOG members prefer the CE regimen over the SPE regimen and consider it to be more suitable for use as a control treatment in future phase III trials.

Compared with the MST obtained for the JCOG 9702 trial (10.6 months for CE versus 9.8 months for SPE), the MST of 13.3 months for the CI regimen in the current study is promising, although the current study included both ED and LD patients as the same population and also included both treated and untreated patients. Furthermore, although 90–95% of the patients in the JCOG 9702 trial experienced grade 3 or 4 neutropenia [25], the toxicity of the current study was 50% and seemed to be generally mild. However, JCOG has also shown that IP is more effective than PE for treating non-elderly patients with ED-SCLC in a phase III trial [6]. Taking these findings together, we are now considering a comparative trial of CE versus CI in elderly patients with ED-SCLC.

In conclusion, the CI regimen was an effective and nontoxic regimen in elderly patients with SCLC, and should be evaluated in future phase III trials.

Acknowledgements

Supported in part by Grants-in-Aid for Cancer Research and for the Second-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labor, and Welfare (Tokyo).

References

[1] Morita T. A statistical study of lung cancer in the annual of pathological autopsy cases in Japan, from 1958 to 1997, with reference to time trends of lung cancer in the world. Jpn J Cancer Res 2002;93:15–23.

[2] Yoshimi I, Ohshima A, Ajiki W, Tsukuma H, Sobue T. A comparison of trends in the incidence rate of lung cancer by histological type in the Osaka cancer registry, Japan and in the surveillance, epidemiology and end results program, USA. Jpn J Clin Oncol 2003;33:98–104.

[3] Kaneko S, Ishikawa KB, Yoshimi I, Marugame T, Hamashima C, Kamo K, et al. Projection of lung cancer mortality in Japan. Cancer Sci 2003;94:919–23.

[4] Fukuoka M, Furuse K, Saijo N, Nishiwaki Y, Ikegami H, Tamura T, et al. Randomized trial of cyclophosphamide, doxorubicin, and vincristine versus cisplatin and etoposide versus alternation of these regimens in small cell lung cancer. J Natl Cancer Inst 1991;83:855–61.

[5] Roth BJ, Johnson DH, Einhorn LH, Schacter LP, Cherng NC, Cohen HJ, et al. Randomized study of cyclophosphamide, doxorubicin, and vincristine versus etoposide and cisplatin versus alternation of these two regimens in extensive small cell lung cancer: a phase III trial of the Southeastern Cancer Study Group. J Clin Oncol 1992;10:282–91.

[6] Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small cell lung cancer. New Engl J Med 2002;346:85–91.

[7] Okamoto H, Watanabe K, Nishiwaki Y, Mori K, Kurita Y, Hayashi I, et al. Phase II study of area under the plasma-concentration-versus-time curve-based carboplatin plus standard-dose intravenous etoposide in elderly patients with small cell lung cancer. J Clin Oncol 1999;17:3540–5.

[8] Okamoto H, Nagatomo A, Kunitoh H, Kunikane H, Watanabe K. A phase I clinical and pharmacologic study of a carboplatin and irinotecan regimen combined with recombinant human granulocyte-colony stimulating factor in the treatment of patients with advanced non-small cell lung carcinoma. Cancer 1998;82:2166–72.

[9] Calvert AH, Newell DR, Gumbrell LA, O'Reilly S, Burnell M, Boxall FE, et al. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J Clin Oncol 1989;7:1748–56.

[10] Abigerges D, Armand JP, Chabot GG, Costa LD, Fadel E, Cote C, et al. Irinotecan (CPT-11) high-dose escalation using intensive high-dose loperamide to control diarrhea. J Natl Cancer Inst 1994;86:446–9.

[11] World Health Organization. WHO handbook for reporting results of cancer treatment. Geneva, Switzerland: Word Health Organization; 1979 (WHO Offset Publication No. 48).

[12] Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.

[13] Medical Research Council Lung Cancer Working Party. Comparison of oral etoposide and standard intravenous multidrug chemotherapy for small cell lung cancer: a stopped multicentre randomized trial. Lancet 1996;348:563–6.

[14] Souhami RL, Spiro SG, Rudd RM, Ruiz de Elvira MC, James LE, Gower NH, et al. Five-day oral etoposide treatment for advanced small cell lung cancer: randomized comparison with intravenous chemotherapy. J Natl Cancer Inst 1997;89:577–80.

[15] Pfeiffer P, Rytter C, Madesen EL, Moeholt K, Hansen O, Bentzen S, et al. Five-day oral etoposide treatment for advanced small cell lung cancer: randomized comparison with intravenous chemotherapy. J Natl Cancer Inst 1997;89:1892–3.

[16] Ardizzoni A, Favaretto A, Boni L, Baldini E, Castiglioni F, Antonelli P, et al. Platinum-etoposide chemotherapy in elderly patients with small cell lung cancer: results of a randomized multicentre phase II study assessing attenuated-dose or full-dose with lenograstim prophylaxis—a Forza Operativa Nazionale Italiana Carcinoma Polmonare and Gruppo Studio Tumori Polmonari Veneto (FONICAP-GSTPV) study. J Clin Oncol 2005;23:569–75.

[17] Fukuda M, Oka M, Soda H, Terashi K, Kawabata S, Nakatomi K, et al. Phase I study of irinotecan combined with carboplatin in previously untreated solid cancers. Clin Cancer Res 1999;5:3963–9.

[18] Sato M, Ando M, Minami H, Ando Y, Ando M, Yamamoto M, et al. Phase I/II and pharmacologic study of irinotecan and carboplatin for patients with lung cancer. Cancer Chemother Pharmacol 2001;48:481–7.

[19] Schmittel A, Schulze K, Hutter G, Krebs P, Thiel E, Keilholz U, et al. Phase I dose escalation study of carboplatin to a fixed dose of irinotecan as first-line treatment of small cell lung cancer. Onkologie 2004;27:280–4.

[20] Findlay MP, Griffin AM, Raghavan D, McDonald KE, Coates AS, Duval PJ, et al. Retrospective review of chemotherapy for small cell lung cancer in the elderly: dose the end justify the means? Eur J Cancer 1991;27:1597–601.

[21] Clamon GH, Audeh MW, Pinnick S. Small cell lung carcinoma in the elderly. J Am Geriat Soc 1982;30:299–302.

[22] Radford JA, Ryder WD, Dodwell D, Anderson H, Thatcher N. Predicting septic complications of chemotherapy: an analysis of 382 patients treated for small cell lung cancer without dose reduction after major sepsis. Eur J Cancer 1992;29A: 81–6.

[23] Timmer-Bonte JN, de Boo TM, Smit HJ, Biesma B, Wilschut FA, Cheragwandi SA, et al. Prevention of chemotherapy-induced febrile neutropenia by prophylactic antibiotics plus or minus granulocyte colony-stimulating factor in small cell lung cancer: a Dutch randomized phase III study. J Clin Oncol 2005;23:7974–84.

[24] Ozer H, Armitage JO, Bennett CL, Crawford J, Demetri GD, Pizzo PA, et al. 2000 update of recommendations for the use of hematopoietic colony-stimulating factors: evidence-based, clinical practice guidelines. J Clin Oncol 2000;18: 3558–85.

[25] Okamoto H, Watanabe K, Kunikane H, Yokoyama A, Kudoh S, Ishizuka N, et al. Randomized phase III trial of carboplatin plus etoposide vs. split doses of cisplatin plus etoposide in elderly or poor-risk patients with extensive disease small cell lung cancer (ED-SCLC): JCOG 9702. J Clin Oncol 2005;23(16S, Part II of II):1094S (late breaking abstract).

Respirology (2006) **11**, 782–785 doi: 10.1111/j.1440-1843.2006.00928.x

ORIGINAL ARTICLE

Transbronchial needle aspiration cytology of subcarinal lymph nodes for the staging procedure in the diagnosis of lung cancer

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Transbronchial needle aspiration cytology of subcarinal lymph nodes for the staging procedure in the diagnosis of lung cancer
AONO H, OKAMOTO H, KUNIKANE H, NAGATOMO A, WATANABE K, NAGAI A. *Respirology* 2006; **11**: 782–785

Objective and background: The aim of this study was to improve the staging of lung cancer with or without lymphadenopathy on chest CT by using transbronchial aspiration cytology (TBAC).
Methods: TBAC of the subcarinal lymph nodes was performed on 153 consecutive patients with lung cancer, with or without subcarinal lymphadenopathy on chest CT.
Results: Thirty-four patients had enlargement of the subcarinal lymph nodes (>1 cm). Eighteen of these had TBAC confirmation of metastases. Another seven patients with no mediastinal involvement on CT were positive for metastases on TBAC. TBAC was the only way to confirm lung cancer in two patients. Therefore, routinely performed subcarinal TBAC contributed to an improved non-operative staging of the patients and diagnosis in 16% (25/153) of the patients with lung cancer. Forty-nine patients with NSCLC had surgical resection of the tumour. Surgical procedure revealed metastases to the subcarinal lymph nodes in three patients in whom the preoperative TBAC diagnosis was normal. No significant complications due to TBAC occurred in any of the patients.
Conclusion: TBAC of the subcarinal lymph nodes is a minimally invasive technique for staging of lung cancer and can provide useful information for the diagnosis of metastases to the subcarinal lymph nodes.

Key words: chest computed tomography, lung cancer, staging, subcarinal lymph node, transbronchial aspiration cytology.

INTRODUCTION

The efficacy of flexible bronchoscopy used in combination with transbronchial needle aspiration (TBNA) has been studied since the early 1980s. TBNA is also known as Wang needle aspiration, and can be performed safely with little morbidity.[1,2] TBNA is most frequently used for cytological diagnosis not only of the parenchymal nodules but also of the mediastinal lymph nodes. Shure and Fedullo reported that TBNA, when used to obtain diagnostic and staging information for mediastinal and subcarinal lymphadenopathy, showed a lower complication rate than mediastinoscopic examination.[3,4] TBNA has become a standard evaluation technique for suspected metastases involving the mediastinal nodes.

Transbronchial aspiration cytology (TBAC) of the subcarinal nodes was performed routinely so as to improve the staging procedure in lung cancer, with or without lymphadenopathy on chest CT. Cytological proof of metastases in the mediastinal lymph nodes and more accurate staging by TBAC.[5] Routinely performed TBAC for subcarinal lymph nodes and optional TBAC of other swollen mediastinal lymph nodes can result in a more correct staging and diagnosis in 25% of patients with lung cancer.[5] In the present study, we analyse how TBAC of subcarinal nodes using flexible bronchoscopy contributes to a

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Received 29 November 2005; invited to revise 5 January and 2 April 2006; revised 12 March and 28 April 2006; accepted 15 May 2006 (Associate Editor: Kwun Fong).

more accurate staging by proving whether N2 disease, according to International Union Against Cancer (UICC) staging,[6] exists or not.

METHODS

Patients

Transbronchial aspiration cytology was performed on 153 consecutive patients with suspected lung cancer during initial diagnostic bronchofibrescopy over an 18-month period. All patients had histological or cytological confirmation of lung cancer after flexible bronchoscopy. Twenty-six patients had small cell lung cancer (SCLC) and 127 had non-small cell lung cancer (NSCLC).

Equipment

The flexible bronchoscope used in the present study was an Olympus (Tokyo, Japan) 1P10 type. The disposable cytology needle used for TBAC was an Olympus 21-gauge, with a length of 15 mm.

Procedure of bronchoscopic examination

As pre-medication, the patients received a 4% solution of nebulized lidocaine and the larynx was anaesthetized with a 2% solution of lidocaine. They were also administered an i.m. injection of atropine sulphate to reduce bronchial secretion. In all cases, a flexible bronchoscope was passed through an endotracheal tube. Prior to oral intubation, the patients were sedated with i.v. administration of diazepam and fentanyl citrate. During these procedures, patients were supplied with oxygen through an endotracheal tube, and fentanyl citrate was administered every 20 min. N-allylnoroxymorphone was given after the procedure was completed.

Transbronchial aspiration cytology was routinely performed on all patients who were suspected of having lung cancer. In order to avoid contamination, TBAC was performed before endobronchial observation and peripheral sampling. Triple punctures in each of the anterior, central and posterior portions of the carina were done to improve diagnostic accuracy with real time X-ray guidance. Once inserted, the needle was moved up and down while syringe suction was maintained.[7] Specimens were sprayed onto glass slides with a 20-mL syringe including air and fixed with 95% ethyl alcohol. We did not perform subcarinal TBAC on patients who had severe chronic pulmonary emphysema or enlargement of the left atrium of the heart, or who were on anticoagulant therapy.

RESULTS

The histological subtypes of the 153 patients enrolled in the study are listed in Table 1. The number of patients who had subcarinal node enlargement >1 cm in short axis diameter on CT (CT-positive) and who had cytological confirmation of metastases by TBAC (TBAC-positive) was 34 (nine SCLC and 25 NSCLC) and 25 (10 SCLC and 15 NSCLC), respectively (Table 2).

The relationship between the size of the subcarinal nodes and result of TBAC is shown in Table 3. Out of 34 CT-positive patients, 18 had confirmed metastases by TBAC. Patients with SCLC had increased TBAC-detection of metastases when they had enlargement

Table 1 Histology of lung cancer in 153 patients who had TBAC

SCLC	26
NSCLC	127
Adenocarcinoma	72
Squamous cell carcinoma	33
Large cell carcinoma	11
Others	11

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; TBAC, transbronchial aspiration cytology.

Table 2 Number of patients who had enlargement of subcarinal nodes (CT-positive) and cytological confirmation of metastasis by TBAC (TBAC-positive)

	CT-positive	TBAC-positive
SCLC	9/26 (35%)	10/26 (38%)
NSCLC	25/127 (20%)	15/127 (12%)
Total	34/153 (22%)	25/153 (16%)

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; TBAC, transbronchial aspiration cytology.

Table 3 Relationship between enlargement of the subcarinal nodes and result of TBAC

	CT-positive	CT-negative
SCLC ($n=26$)		
TBAC-positive	7	3
TBAC-negative	2	14
NSCLC ($n=127$)		
TBAC-positive	11	4
TBAC-negative	14	98
Total ($n=153$)		
TBAC-positive	18	7
TBAC-negative	16	112

CT-negative, patients without enlargement of the subcarinal nodes; CT-positive, patients with enlargement of the subcarinal nodes; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; TBAC, transbronchial aspiration cytology; TBAC-negative, patients who did not have confirmation of metastasis to the subcarinal nodes by TBAC; TBAC-positive, patients who had confirmation of metastasis to the subcarinal nodes by TBAC.

Table 4 Relationship between the site of primary tumour and CT findings or results of TBAC (n=153)

Primary site	No. patients	CT-positive	CT-negative	TBAC-positive	TBAC-negative
LUL	42	9	33	5	37
LLL	17	7	10	4	13
LMB	4	1	3	2	2
RUL	34	8	26	3	31
RML	11	3	8	3	8
RLL	35	3	32	4	31
RMB	1	1	0	1	0
Intermedius	5	2	3	2	3
Unknown	4	0	4	1	0
Total	153	34	119	25	128

LLL, left lower lobe; LMB, left main bronchus; LUL, left upper lobe; RLL, right lower lobe; RMB, right main bronchus; RML, right middle lobe; RUL, right upper lobe; TBAC, transbronchial aspiration cytology.

of the nodes (7/9) than ones with NSCLC (11/25). Out of 119 patients without enlargement of the subcarinal nodes (CT-negative), TBAC did not reveal metastases (TBLB-negative) in 112, but seven patients had confirmed metastases by TBAC. The lymphoid cells of TBAC samples were obtained in 112 (79%) of 153 cases.

Forty-nine patients with NSCLC had surgical resection of the tumour. There were no resected cases who were TBAC-positive. In our hospital, pathologically confirmed N2 disease was considered inoperable even though there was no enlargement of mediastinal lymph node on chest CT scan. Furthermore, during the study period, no clinical trials such as neoadjuvant chemotherapy followed by surgery or surgery after adjuvant chemotherapy were available for pathological confirmed N2 disease in our hospital. Therefore, seven patients with pathologically confirmed N2 were treated with radiotherapy with/without chemotherapy. The surgical procedure revealed metastases to the subcarinal nodes in three patients, although preoperative TBAC diagnosis did not show any metastases. All three p-N2 patients who had negative TBAC showed an absence of subcarinal lymph nodes swelling on preoperative chest CT scan. The other 46 patients who had negative subcarinal nodes biopsy by TBAC showed no metastases in resected specimens. The accuracy of TBAC for diagnosing metastases was 94% in the 49 patients. The relationship of the site of primary tumour and CT findings or results of TBAC is listed in Table 4. No exact correlation was observed between the site of primary tumour and the results of TBAC. Summary of the patients in which subcarinal TBAC contributed to the staging or diagnosis are as follows. Radiological N2 was positively confirmed by subcarinal TBAC in 18 patients. N2 was confirmed by subcarinal TBAC in the absence of subcarinal lymph nodes swelling in seven patients. Subcarinal TBAC was the only way to confirm lung cancer in two patients. Therefore, routinely performed subcarinal TBAC contributed to more correct staging and diagnosis in 16% of the patients with lung cancer. No severe complications occurred in any of the cases who received routinely performed subcarinal TBAC.

DISCUSSION

Accurate diagnosis of metastases to the mediastinal lymph nodes influences the treatment plan and prognosis of patients with lung cancer.[8] As approximately 30–40% of patients with lung cancer already have mediastinal metastases at the time of initial diagnosis,[9] and histological or cytological evaluation of metastases to the mediastinal nodes is essential.

Generally, diagnosis of metastases to the mediastinal lymph nodes is based upon imaging and histological information. Commonly used imaging equipment includes positron emission tomography (PET), magnetic resonance imaging and CT. In most clinical settings, contrast-enhanced CT is the investigation of choice, and the size of lymph nodes provides a standard for the diagnosis of metastases by CT.[9] However, micrometastases could be present in lymph nodes without node enlargement and equally enlarged nodes may be due entirely to inflammation.[10] The relationship between size of lymph nodes and presence of malignancy is highly variable. The diagnosis of mediastinal lymph node metastases by CT is based solely on size with the cut-off value being >1.0 cm on the short axis diameter. Mediastinoscopy, video-assisted thoracoscopic surgery and TBAC are used as invasive diagnostic procedures for the sampling of lymph node cells, but TBAC can be performed with relatively simple anaesthesia in a bronchoscopic examination.

Our study showed that TBAC confirmed metastases in 42% of cases with enlargement of the subcarinal nodes. This detection rate was lower than in previous reports, although a high detection (7/9) rate was achieved in patients with SCLC, consistent with previous reports.[7,8] One of the possible reasons for this low rate was that TBAC was performed only on subcarinal nodes, while TBAC was performed at multiple sites in other reports.[7,8] Accuracy of TBAC could not be assessed in the present study because metastases was not finally diagnosed in the TBAC-negative cases, and this is one of the study's limitations. Another limitation is that TBAC is a blind technique with guidance limited to a few endobronchial landmarks and mental reconstruction of the CT scan. We operated on 49

patients with NSCLC and subcarinal metastases was found in three patients by postoperative pathological assessment. The accuracy of TBAC was 94% in the operated patients, which showed the limit of TBAC in establishing a diagnosis. It is possible that the TBAC needle used in this study may not collect enough cells for assessment and would suggest our method might be less useful for identifying micrometastases of lymph nodes. Furthermore, lymphoid cells were obtained in only 112 (79%) of 153 cases. In other words, TBAC could not adequately sample the target lymph nodes in 21% of patients.

In operable cases, right upper lobe tumours might be more likely to spread to the paratracheal region than to the subcarinal region. However, as shown in Table 4, no exact correlation was observed between the site of primary tumour and the TBAC results. This may be due to the fact that more patients with advanced stage tumour were included and only 49 of 153 patients had surgery in our study.

Recent studies for the diagnosis of lung cancer have shown that the highest detection rate of metastases to lymph nodes is achieved by PET,[10] but the role of PET in the treatment plan remains controversial. Mediastinoscopy is usually the best choice for proof of metastases to mediastinal nodes, but it is unable to assess all lymph nodes. TBAC should be performed in combination with other diagnostic procedures. In order to improve the diagnosis by TBAC, TBAC under the guide of CT or endoscopic ultrasound has been developed,[10] although these procedures are still experimental. Metastases to the subcarinal nodes was demonstrated following TBAC in some patients without nodal enlargement. Few studies have been undertaken to assess the presence of metastases in mediastinal lymph nodes that are not enlarged, and TBAC may have diagnostic value in these cases. The potential contribution of the present study is to ask what a blind TBAC in normal sized nodes adds to preoperative staging. Of 119 patients with normal sized nodes there were seven with positive cytology on TBAC. Conversely there were three patients, which were not detected preoperatively in 49 operable patients. Based on the results of the present study, it might be difficult to recommend routine TBAC preoperatively. It was anticipated that analysis of the site of primary tumour might suggest which patients a clinician should have a blind TBAC but the data were not discriminatory as shown in Table 4.

Positron emission tomography is more accurate than CT for detecting mediastinal metastases. However, it should be noted that even PET scan frequently shows false-positive and false-negative in mediastinal staging in the range of 11–16%.[11] Because the detection rate of TBAC using our method was not very high, mediastinoscopy should still be considered the gold standard to confirm N2 disease. Toloza *et al.* reported a meta-analysis of invasive staging consisting of TBAC (TBNA), transtracheal needle aspiration, endoscopic ultrasound-guided needle aspiration and mediastinoscopy. They reported that TBAC has the worst sensitivity and negative predictive value among the invasive procedures.[9] However, considering that TBAC is an easy additional procedure during routine bronchofibreoscopy, the diagnostic yields of TBAC are comparable with other procedures. Furthermore, patients may avoid mediastinoscopy if TBAC is positive, therefore this is useful even if the yield is lower than mediastinoscopy

Transbronchial aspiration cytology of the subcarinal nodes is a minimally invasive technique for staging lung cancer. It can provide useful information for diagnosis of metastases to subcarinal nodes.

REFERENCES

1 Wang KP, Brouser R, Haponik EF *et al.* Flexible transbronchial needle aspiration for staging of bronchogenic carcinoma. *Chest* 1983; **84**: 571–6.
2 Buriski G, Calverley PM, Douglous NJ *et al.* Bronchial needle aspiration in the diagnosis of bronchial carcinoma. *Thorax* 1982; **36**: 508–11.
3 Shure D, Fedllo PF. Transbronchial needle aspiration in the diagnosis of submucosal and peribronchial bronchogenic carcinoma. *Chest* 1985; **88**: 49–51.
4 Shure D, Fedllo PF. The role of transcarinal needle aspiration in the staging of bronchogenic carcinoma. *Chest* 1984; **86**: 693–6.
5 Nagatomo A, Okamoto H, Kunikane H *et al.* Role of TBAC (transbronchial needle aspiration cytology) in the diagnosis of metastases to subcarinal lymph nodes. *J. Jpn. Soc. Resp. Endosc.* 1996; **18**: 837–41 (in Japanese).
6 Mountain CF. Revision in the international system for staging lung cancer. *Chest* 1997; **111**: 1710–17.
7 Dasgupta A, Mehta AC. Transbronchial needle aspiration. An underused diagnostic technique. *Clin. Chest Med.* 1999; **20**: 39–51.
8 Detterbeck FC, DeCamp MM Jr, Kohman LJ, Silvestri GA. American College of Chest Physicians. Lung cancer. Invasive staging: the guidelines. *Chest* 2003; **123** (Suppl. 1): 167S–175S.
9 Toloza EM, Harpole L, Detterbeck F, McCrory DC. Invasive staging of non-small cell lung cancer: a review of the current evidence. *Chest* 2003; **123** (Suppl. 1): 157S–166S.
10 Kramer H, Groen HJ. Current concepts in the mediastinal lymph node staging of non-small cell lung cancer. *Ann. Surg.* 2003; **238**: 180–8.
11 Toloza EM, Harpole L, McCrory DC. Noninvasive staging of non-small cell lung cancer: a review of the current evidence. *Chest* 2003; **123** (Suppl. 1): 137S–146S.

JOURNAL OF CLINICAL ONCOLOGY 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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Submitted June 24, 2003; accepted November 7, 2003.

Supported by a grant from Aventis Pharma Ltd, Tokyo, Japan.

Previously presented in part at the Annual Meetings of the American Society of Clinical Oncology, San Francisco, CA, May 12-15, 2001, and Orlando, FL, May 18-21, 2002.

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

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0732-183X/04/2202-254/$20.00

DOI: 10.1200/JCO.2004.06.114

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.

J Clin Oncol 22:254-261. © 2004 by American Society of Clinical Oncology

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