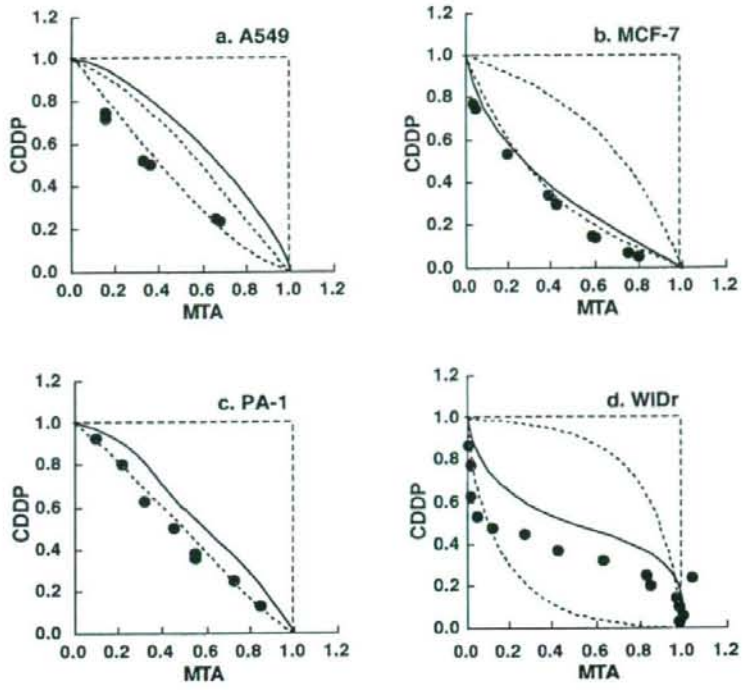

Figure 4. Isobolograms of sequential exposure to pemetrexed (24 h) followed by cisplatin (24 h) in A549 (a), MCF7 (b), PA1 (c), and WiDr (d) cells. For the A549, MCF7, and PA1 cells, all or most of the data points of the combinations fell within the envelope of additivity and in the area of supra-additivity.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

RESULTS

The IC_{80} values of 24-h exposure to pemetrexed for A549, MCF7, PA1, and WiDr cells were >5, 2.5 ± 0.4, 0.10 ± 0.03, and 0.55 ± 0.2 μM, respectively. Because A549 cells were resistant to pemetrexed and the IC_{80} level was not obtained, the interactions between pemetrexed and cisplatin were evaluated at the IC_{50} level. The IC_{50} value of 24-h exposure to pemetrexed for A549 cells was 2.7 ± 0.3 μM.

Figure 2 shows the dose–response curves obtained from simultaneous exposure and sequential exposure to pemetrexed and cisplatin for the PA1 cells. The dose–response curves were plotted on a semilog scale as a percentage of the control, the cell number of which was obtained from the samples not exposed to the drugs administered simultaneously. Dose–response curves in which the pemetrexed 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 cisplatin alone, three isoeffect curves (mode I and mode II lines) were constructed. Isobolograms at the IC_{80} or IC_{50} levels were generated based upon these dose–response curves for the combinations.

Simultaneous Exposure to Pemetrexed and Cisplatin

Figure 3 shows isobolograms of the A549, MCF7, PA1, and WiDr cells after simultaneous exposure to pemetrexed and cisplatin for 24 h. For the A549, MCF7, and PA1 cells, the combined data points fell in the areas of subadditivity and protection, respectively. The mean values of the observed data (>1.15, 0.95, and 0.69) were larger than those of the predicted maximum values (0.75, 0.72, and 0.56). The observed data and the predicted maximum data were compared by the Wilcoxon signed-rank test. The differences were significant ($p < 0.05$, $p < 0.02$, and $p < 0.01$), indicating antagonistic effects (Table 1). For the WiDr cells, the combined data points fell mainly within the envelope of additivity. The mean values of the observed data (0.66) were larger than those of the predicted minimum values (0.27), and smaller than those of the predicted maximum values (0.73), indicating additive effects.

Sequential Exposure to Pemetrexed Followed by Cisplatin

Figure 4 shows isobolograms of the A549, MCF7, PA1, and WiDr cells exposed first to pemetrexed for 24 h and then cisplatin for 24 h. For the MCF7 cells, combined data points fell in the area of supra-additivity. The mean values of the observed data (0.40) were smaller than those of the predicted minimum values (0.44) (Table 1). The difference between them was significant ($p < 0.01$), indicating synergistic effects. For the A549 and PA1 cells, combined data points fell in the area of supra-additivity and within the envelope of additivity. The mean values of the observed data were smaller than those of the predicted minimum values (Table 1), but the differences were not significant ($p > 0.05$ and $p > 0.05$), indicating additive/synergistic effects. For the WiDr cells, the combined data points fell within the envelope of additivity and in the areas of supra-additivity and protection. The mean value of the observed data was smaller than the predicted maximum values and larger than that of the predicted minimum values (Table 1), indicating additive effects.

Sequential Exposure to Cisplatin Followed by Pemetrexed

Figure 5 shows isobolograms of the four cell lines exposed first to cisplatin for 24 h and then pemetrexed for 24 h. For the A549, MCF7, and PA1 cells, all or most of the combined data points fell in the areas of subadditivity and protection. The mean values of the observed data were larger than those of the predicted maximum values (Table 1). The differences were significant ($p < 0.05$, $p < 0.02$, and $p < 0.02$, respectively), indicating antagonistic effects. For the WiDr cells, most of the combined data points fell within the envelope of additivity, indicating an additive effect of this schedule.

Flow Cytometric Analysis

Finally, we evaluated the cytotoxic effects of pemetrexed and cisplatin on cancer cells using flow cytome-

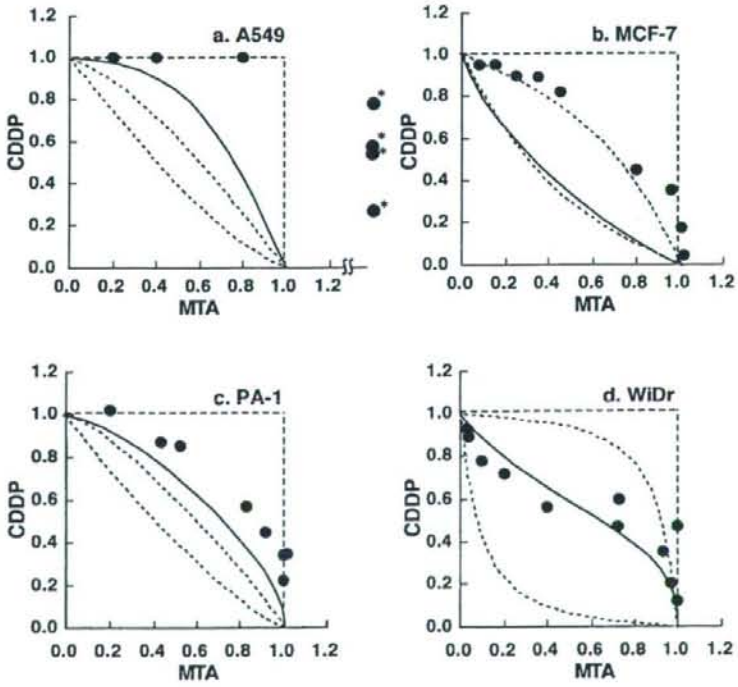

Figure 5. Isobolograms of sequential exposure to cisplatin (24 h) followed by pemetrexed (24 h) in A549 (a), MCF7 (b), PA1 (c), and WiDr (d) cells. For the A549, MCF7, and PA1 cells, all or most of the data points of the combinations fell in the areas of subadditivity and protection. For the WiDr cells, most of the data points of the combinations fell within the envelope of additivity and in the area of subadditivity. Data are mean values for at least three independent experiments; SE was <20%(*except the data).

Table 1. Mean Values of Observed, Predicted Minimum, and Predicted Maximum Data of Pemetrexed (MTA) in Combination With Cisplatin (CDDP) at IC_{80} for MCF7, PA1, and WiDr Cells and at IC_{50} for A549 Cells

Schedule	Cell Line	n	Observed Data	Predictaed Data for an Additive Effect		Effect
				Minimum	Maximum	
MTA + CDDP	A549	6	1.15	0.44	0.75	antagonism ($p < 0.05$)
	MCF7	8	0.95	0.57	0.72	antagonism ($p < 0.02$)
	PA1	9	0.69	0.40	0.56	antagonism ($p < 0.01$)
	WiDr	9	0.66	0.27	0.73	additive
MTA → CDDP	A549+	6	0.45	0.47	0.72	additive/synergism ($p > 0.05$)
	MCF7	9	0.40	0.44	0.78	synergism ($p < 0.01$)
	PA1	8	0.52	0.55	0.64	additive/synergism($p > 0.05$)
	WiDr	15	0.64	0.46	0.84	additive
CDDP → MTA	A549	7	1.14	0.41	0.74	antagonism ($p < 0.05$)
	MCF7	9	0.82	0.52	0.73	antagonism ($p < 0.02$)
	PA1	8	0.75	0.41	0.63	antagonism ($p < 0.02$)
	WiDr	11	0.71	0.21	0.82	additive

try. Cell cycle analysis revealed that pemetrexed and cisplatin arrested PA1 cells in late G_1 to early S phase and G_2/M phase, respectively (Fig. 6A, Table 2). When PA1 cells were exposed to both drugs simultaneously, the cell cycle profile was almost identical to that of a single treatment with pemetrexed, suggesting that the cell cycle effect of pemetrexed is dominant over that of cisplatin. As a result, the apoptosis-inducing effect of cisplatin, which was estimated by an increase in the size of sub-G_1 fraction, was almost completely cancelled in the presence of pemetrexed (Fig. 6A, MTA + CDDP). When PA1 cells were treated with cisplatin first and followed by pemetrexed, the cell cycle pattern closely resembled that of cells treated with cisplatin alone except for a modest increase in G_1 and S phases (Fig. 6A, Table 2, CDDP to MTA). The induction of apoptotsis was less prominent in the CDDP to MTA treatment than in the CDDP treatment (Table 2). In contrast, both apoptosis and G_2/M arrest were enhanced when PA1 cells were treated with pemetrexed first and followed by cisplatin compared with the treatment with either pemetrexed or cisplatin alone (Fig. 6A, Table 2, MTA to CDDP).

We carried out the same analysis with another cancer cell line MCF7 and obtained highly reproducible results. Upon simultaneous addition, the cell cycle effect of cisplatin was almost completely abrogated and the percentage of apoptotic cells was less than that of a single treatment with pemetrexed (Fig. 6B, MTA + CDDP). Similarly, apoptosis was suppressed when MCF7 cells were treated with cisplatin first and followed by pemetrexed compared with the treatment with either pemetrexed or cisplatin alone (Fig. 6B, Table 2, CDDP to MTA). In contrast, the apoptosis-inducing effect of pemetrexed was enhanced by the sequential exposure to cisplatin after pemetrexed (Fig. 6B, Table 2, MTA to CDDP). Overall, these data are fully consistent with the results of isobologram analysis, and provide the molecular basis of the interaction between the two drugs.

DISCUSSION

We found that the cytotoxic interaction between pemetrexed and cisplatin was schedule dependent. Simultaneous exposure to pemetrexed and cisplatin and sequential exposure to cisplatin followed by pemetrexed showed antagonistic effects in A549, MCF7, and PA1 cells, while sequential exposure to pemetrexed followed by cisplatin had a tendency to produce synergistic effects. In the latter schedule, observed data points in A549, MCF7, and PA1 cells were smaller than predicted minimum values for an additive effect (Table 1). WiDr cells showed additive effects in all schedules. The cause of difference in combined effects among cell lines is unknown. The difference may reflect the folate metabolism and the variety of target numbers (enzymes) in the cells. In addition, the isobologram of Steel and Peckham is stricter for synergism and antagonism than other methods. This may also influence the results.

In general, it is difficult to clarify the mechanisms underlying the drug combination. In this study, however, cell cycle analysis provided a clue to understand the molecular basis of schedule-dependent synergism and antagonism of the combination of pemetrexed and cisplatin. The exposure of PA1 and MCF7 cells to pemetrexed for 24 h led to a synchronization of most cells in late G_1 to

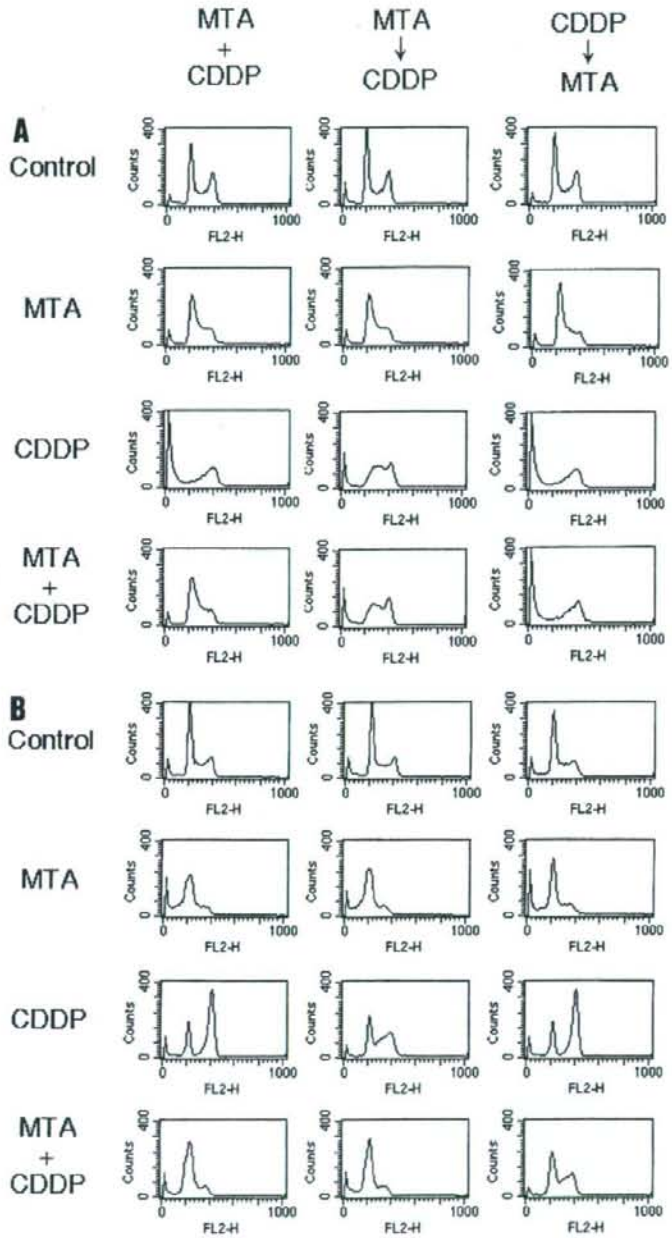

Figure 6. Flow cytometric analysis of cell cycle perturbation. PA1 cells, treated with 0.2 μM pemetrexed (MTA), 0.5 μM cisplatin (CDDP), both drugs simultaneously for 24 h, pemetrexed for 24 h followed by cisplatin for 24 h, or the reverse sequence were harvested at 48 h (A), and MCF7 cells, treated with 0.5 μM pemetrexed (MTA), 5 μM cisplatin (CDDP), both drugs simultaneously for 24 h, pemetrexed for 24 h followed by cisplatin for 24 h, or the reverse sequence were harvested at 48 h (B) and stained for DNA with propidium iodide and analyzed by flow cytometry as described in Materials and Methods.

Table 2. Cell Cycle Perturbations Induced by Pemetrexed (MTA), Cisplatin (CDDP), and Their Combinations for PA1 and MCF7 Cells at 48 h

	MTA + CDDP (24 h)				MTA (24 h) → CDDP (24 h)				CDDP (24 h)→ MTA (24 h)			
Cell Cycle (%)	Control	MTA	CDDP	MTA + CDDP	Control	MTA	CDDP	MTA + CDDP	Control	MTA	CDDP	MTA + CDDP
PA1 cells												
Sub-G_1	3.6	2.4	42.9	2.1	4.3	3.1	8.9	15.3	2.9	2.2	45.1	41.8
G_1	56.2	64.1	7.3	67.1	58.1	65.3	5.8	4.4	57.3	60.1	6.9	10.6
S	15.6	26.7	17.2	19.1	10.4	25.9	48.4	38.7	11.0	30.4	15.8	20.1
G_2/M	24.6	6.8	19.1	11.7	27.2	5.7	36.9	41.6	28.8	7.3	32.2	27.5
MCF-7 cells												
Sub-G_1	4.2	17.5	3.9	5.8	5.3	11.1	2.9	16.8	5.1	10.3	3.6	2.5
G_1	57.6	53.4	28.8	63.7	55.8	61.3	22.3	60.6	58.8	57.2	27.9	25.8
S	16.8	26.9	4.7	21.4	19.1	22.1	21.2	13.8	16.4	28.6	5.0	20.4
G_2/M	21.4	2.2	62.6	9.1	25.1	5.5	53.6	8.8	19.7	3.9	63.5	51.3

early S phase, in which cells are sensitive to cisplatin (20). This may explain the synergistic effects of sequential exposure to pemetrexed followed by cisplatin. On the contrary, one agent may 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. It has been shown that cisplatin elicits cytotoxic effects by blocking cells in G_2/M phase (20), while pemetrexed does by blocking cells in S phase (21). Indeed, simultaneous exposure to pemetrexed and cisplatin produced antagonistic effects, which were caused by the cancellation of cisplatin-induced G_2/M arrest by coexisting pemetrexed in PA1 and MCF7 cells. This was also the case with sequential exposure with cisplatin first followed by pemetrexed.

Our findings suggest that the sequential administration of pemetrexed followed by cisplatin may be the optimal schedule for these combinations. For example, administrations of pemetrexed on day 1 and cisplatin on day 2 would be worthy of clinical investigations. The simultaneous administration of pemetrexed and cisplatin and the sequential administration of cisplatin followed by pemetrexed may be inadequate. However, it must be noted that there are a number of difficulties in the translation of results from in vitro models to clinical therapy. The drug metabolism and pharmacokinetics under in vivo and in vitro conditions are different. Clinical outcome includes both the antitumor effects and normal tissue toxicity that results from a variable drug exposure, whereas in vitro models represent only antitumor effects at a constant drug exposure.

Teicher et al. studied the combination of pemetrexed with cisplatin in vivo against EMT-6 murine mammary carcinoma by a tumor cell survival assay (26). They observed that pemetrexed administered four times over 48 h with cisplatin administered with the third dose of pemetrexed produced an additive or more than additive tumor response. Teicher et al. further studied the combination of pemetrexed with cisplatin in human tumor xenografts (27). Administration of pemetrexed (days 7–11, days 14–18) along with cisplatin (day 7) produced greater-than-additive effects for human lung cancer H460 and Calu-6 tumor growth delay. Because experimental systems, schedules of drug administrations, and evaluating methods for synergism are different, it is difficult to compare their findings and ours.

A clinical and pharmacokinetic phase I study of pemetrexed in combination with cisplatin has been reported by Thordtmann et al. (15). They observed that this combination was clinically active and simultaneous administration of both agents on day on 1 (pemetrexed intravenously over 10 min and cisplatin over 2 h) every 21 days was less toxic than a sequential administration of pemetrexed on day 1 and cisplatin on day 2. They recommended the simultaneous administration of pemetrexed at 500 mg/m^2 plus cisplatin at 75 mg/m^2 on day 1 every 21 days for this combination. Phase II and III studies of the same schedules have been started for this combination and encouraging results have been obtained so far (16–18).

Our in vitro findings are not contradictory to clinical findings. In our study, simultaneous exposure to pemetrexed and cisplatin produced additive effects in WiDr cells and antagonistic effects in A549, MCF7, andPA1 cells. Most data points fell in the area of sudadditivity in MCF7 and PA1 cells, suggesting that the combination is superior to each drug alone but "sub-optimal." The simultaneous administration of pemetrexed and cisplatin was less toxic than the sequential administration, probably due to antagonistic interaction in the simultaneous exposure. Our isobologram shows that the doses of both agents in the pemetrexed–cisplatin sequence required

for IC_{80} or IC_{50} levels were much less (40–90%) than of those in simultaneous exposure (Fig. 3). Pemetrexed at 500 mg/m² and cisplatin at 75 mg/m², the optimal dose for the simultaneous administration, would be overdosed for the sequential administration of pemetrexed followed by cisplatin, which produced synergistic effects.

In conclusion, the present findings show that the interaction of pemetrexed and cisplatin is definitely schedule dependent. Sequential exposure to pemetrexed followed by cisplatin produced synergistic effects, whereas simultaneous exposure to the two agents and sequential exposure to cisplatin followed by pemetrexed produced antagonistic effects. These findings suggest that the optimal schedule of pemetrexed in combination with cisplatin at the cellular level is the sequential administration of pemetrexed followed by cisplatin. Although the simultaneous administration of pemetrexed and cisplatin on day 1 is more convenient and less toxic for patients than the sequential administration of pemetrexed on day 1 and cisplatin on day 2, the former schedule may be suboptimal and may not improve the clinical efficacy to "originally expected" level for this combination. It would be important to conduct dose-finding clinical trials in sequential administration of pemetrexed and cisplatin.

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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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KEYWORDS
Small cell lung cancer;
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Carboplatin;
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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

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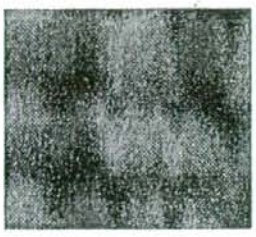

received two or more cycles of chemotherapy, and the median actual delivery of irinotecan was 84% of the projected dose. Grade 3/4 neutropenia, anemia, and diarrhea occurred in 50%, 33% and 6% of patients, respectively. Other toxicities were mild and no treatment-related deaths occurred. The response 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

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/m²)
1	4		50
2	5		50
3	5		60

AUC, area under the curve.

(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).

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
2–3	10	29 (25–36)	0
3–4	9	28 (27–35)	0

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	7	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 Nonhematologic toxicity (worst of any course)

Level	No. of patients	Nausea/vomiting			Diarrhea			Infection			Pulmonary			Liver		Renal	
		Grade 1	Grade 2	Grade ≥3	Grade 1	Grade 2	Grade ≥3	Grade 1	Grade 2	Grade ≥3	Grade 1	Grade 2	Grade ≥3	Grade 1	Grade ≥2	Grade 1	Grade ≥2
1	6	5	0	1	3	1	0	1	0	0	0	0	0	1	0	0	0
2	7	7	0	0	3	0	0	0	0	0	0	0	0	1	0	0	0
3	2	2	0	0	1	0	0	1	0	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	6	6	1	5	0	0
2	10	3	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

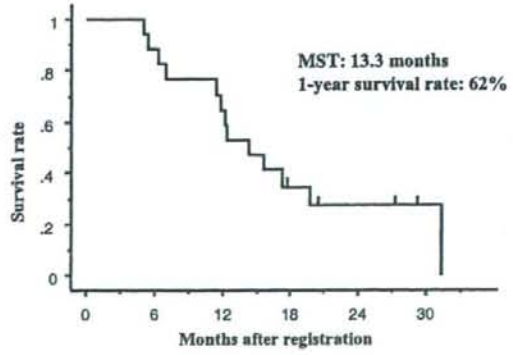

Fig. 1 Overall survival curve.

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 non-toxic regimen in elderly patients with SCLC, and should be evaluated in future phase III trials.

Acknowledgements

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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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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.

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ORIGINAL REPORT

Phase II Trial of Amrubicin for Treatment of Refractory or Relapsed Small-Cell Lung Cancer: Thoracic Oncology Research Group Study 0301

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ABSTRACT

Purpose
This multicenter, phase II study was conducted to evaluate the activity of amrubicin, a topoisomerase II inhibitor, against refractory or relapsed small-cell lung cancer (SCLC).

Patients and Methods
SCLC patients with measurable disease who had been treated previously with at least one platinum-based chemotherapy regimen and had an Eastern Cooperative Oncology Group performance status of 0 to 2 were eligible. Two groups of patients were selected: patients who experienced first-line treatment failure less than 60 days from treatment discontinuation (refractory group), and patients who responded to first-line treatment and experienced disease progression ≥ 60 days after treatment discontinuation (sensitive group). Amrubicin was administered as a 5-minute daily intravenous injection at a dose of 40 mg/m^2 for 3 consecutive days, every 3 weeks.

Results
Between June 2003 and December 2004, 60 patients (16 refractory and 44 sensitive) were enrolled. The median number of treatment cycles was four (range, one to eight). Grade 3 or 4 hematologic toxicities comprised neutropenia (83%), thrombocytopenia (20%), and anemia (33%). Febrile neutropenia was observed in three patients (5%). Nonhematologic toxicities were mild. No treatment-related death was observed. The overall response rates were 50% (95% CI, 25% to 75%) in the refractory group, and 52% (95% CI, 37% to 68%) in the sensitive group. The progression-free survival, overall survival, and 1-year survival in the refractory group and the sensitive group were 2.6 and 4.2 months, 10.3 and 11.6 months, and 40% and 46%, respectively.

Conclusion
Amrubicin exhibits significant activity against SCLC, with predictable and manageable toxicities; this agent deserves to be studied more extensively in additional trials.

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INTRODUCTION

Approximately 15% of all patients with lung cancer are diagnosed with small-cell lung cancer (SCLC). Unlike other types of lung cancer, SCLC is sensitive to chemotherapy or radiation therapy.[1] Nonetheless, after experiencing an apparently successful induction therapy, most patients experience relapse within 2 years because of the emergence of drug-resistant cancer cells during the induction therapy or the existence of such cells before chemotherapy. Therefore, long-term survival is quite uncommon, with less than 25% of patients with limited-stage, and 1% to 2% of patients with extensive-stage disease remaining alive at 5 years.[2-4] Furthermore, the results of second-line chemotherapy against SCLC are disappointing, with relatively low response rates, brief remissions, and a short survival time.[1,5] In particular, little progress has been made in the re-treatment of patients who experienced progression during first-line therapy or who failed to achieve a progression-free survival of more than 60 to 90 days. As a result, to control SCLC more efficiently, new drugs that are effective for patients who have failed to respond to standard treatment, and who may have multidrug-resistant tumors, are urgently needed.

Amrubicin, a totally synthetic 9-aminoanthracycline, is converted to an active metabolite, amrubicinol, through the reduction of its C-13 ketone group to a hydroxy group.[6] Despite the similarity of its chemical structure to that of a representative anthracycline, doxorubicin, the mode of action of amrubicin differs from that of doxorubicin.[7] Amrubicin and amrubicinol are inhibitors of DNA topoisomerase II, which exert cytotoxic effects by stabilizing a topoisomerase II-mediated cleavable complex, and are approximately 1/10 weaker than doxorubicin as a DNA intercalator. The in vitro cytotoxic activity of amrubicinol was 18 to 220 times more potent than that of its parent compound, amrubicin.[8] In preclinical studies, amrubicin showed a more potent antitumor activity than doxorubicin in several human tumor xenografts implanted in nude mice,[9] and caused almost no cardiotoxicity.[9,10] The response rates to amrubicin at a dose of 45 mg/m^2 on days 1 to 3 in chemotherapy-naive patients with stage III or IV non-SCLC and extensive-stage SCLC were 25% and 79% on an intent-to-treat analysis, respectively.[11,12] The major grade 3 or 4 toxicities were neutropenia (72.1%), leukopenia (52.5%), anemia (23.0%), thrombocytopenia (14.8%), anorexia (4.9%), and nausea/vomiting (4.9%) in a phase II trial.[13]

The high activity of amrubicin as a single agent in untreated patients with extensive disease (ED) SCLC led us to carry out this phase II trial, which was designed to determine the antitumor activity and toxicity of amrubicin in previously treated patients with SCLC.

PATIENTS AND METHODS

Patient Selection

Before participation in the present study, each patient was examined to ensure he or she met the following criteria: histologic or cytologic proof of SCLC; recurrent or refractory disease after one or two previous chemotherapy regimens (at least one platinum-containing regimen); measurable disease; no chemotherapy or chest radiotherapy within 4 weeks before entry (measurable disease outside the radiation field); life expectancy of at least 8 weeks; performance status of 2 or better according to the Eastern Cooperative Oncology Group scale; age ≥ 20 years; adequate bone marrow function (leukocyte count ≥ 4,000/μL, absolute neutrophil count [ANC] ≥ 2,000/μL, platelet count ≥ 100,000/μL, and hemoglobin ≥ 9.0 g/dL) and hepatic function (AST and ALT ≤ 100 U/L, or ≤ 200 U/L in the presence of liver metastases; bilirubin level ≤ 1.5 mg/dL); ECG findings within the normal range, and a left ventricular ejection fraction ≥ 50%; arterial oxygen partial pressure ≥ 60 torr; and the written informed consent of the patient. Patients were ineligible if they had serious infectious diseases or other severe complications (heart disease, pulmonary fibrosis/interstitial pneumonia, or uncontrollable diabetes); had massive pleural or pericardial effusion, or ascitic fluid; had symptomatic brain metastases; had active concurrent malignancies; were lactating or pregnant women or hoped to become pregnant; had a history of a drug allergy; or had other medical problems severe enough to prevent compliance with the protocol. Prior amrubicin chemotherapy was not allowed. Trial document approval was obtained in advance from the ethics committee or institutional review board of each hospital.

Treatment Schedule

Amrubicin was dissolved in 20 mL of normal saline, and administered intravenously as a 5-minute infusion at a dose of 40 mg/m^2/d on days 1 to 3 every 3 weeks. Patients with evidence of disease progression or who experienced intolerable toxicity, such as grade 2 or worse pneumonitis, were removed from the study. Before the next course could be started, the patient's ANC had to be ≥ 1,500/μL, his or her platelet count had to be ≥ 100,000/μL, and any nonhematologic toxicities should have been downgraded to at least grade 1. If more than 6 weeks passed from the time of the last treatment before these criteria were satisfied, the patient was removed from the study.

Granulocyte colony-stimulating factor (G-CSF) was permitted as a therapeutic intervention but was not mandatory as a prophylactic agent against neutropenia for hematologic toxicity.

Subsequent doses were modified based on hematologic and nonhematologic toxicities. If the leukocyte count was less than 1,000/μL for 4 days or longer, the ANC was less than 500/μL for 4 days or longer, the platelet count nadir was less than 20×10^3/μL, or grade 3 or worse nonhematologic toxicity was observed, the dose of amrubicin was reduced to 35 mg/m^2/d. The dose of amrubicin also was reduced to 35 mg/m^2/d in patients who developed grade 3 febrile neutropenia.

Evaluation

Patients were evaluated to determine the stage of disease at the time of disease progression or at the time of relapse by taking a complete medical history and performing a physical examination, chest radiograph, computed tomography of the chest and abdomen, and other staging procedures as indicated, including computed tomography of the head and a bone scintiscan. Limited disease (LD) was defined as that confined to one hemithorax, including bilateral mediastinal and bilateral supraclavicular nodes: any involvement beyond these confines was defined as ED. Primary refractory disease (refractory group) was defined as relapse during the first-line chemotherapy regimen or less than 60 days after completing the initial chemotherapy regimen, and sensitive disease (sensitive group) was defined as relapse ≥ 60 days after completion of the first-line chemotherapy. Before the first course, each patient was assessed using a CBC, including a differential count and a platelet count, and serum chemistry tests for renal and hepatic functions as well as electrolytes. The CBC and biochemistry tests were repeated at least once a week after this initial evaluation, whereas the other investigations were repeated at least every 6 weeks to evaluate the target lesions.

Adverse events were recorded and graded using the National Cancer Institute Common Toxicity Criteria, Version 2.0 grading system. After completing the chemotherapy regimen, each patient was restaged using all of the tests used during the initial work-up. The tumor response was classified in accordance with the Response Evaluation Criteria in Solid Tumors.[14] The duration of the response was defined as the number of days from the documentation of the response to the detection of disease progression. The eligibility, evaluability, and response of each patient were assessed by extramural reviewers. The duration of survival, determined as the number of days between the enrollment of protocol therapy and death, was censored at the time last known alive for patients who had not died.

Statistical Methods

Kaplan-Meier survival estimates were used to summarize the time-to-event variables.[15] These included time to response, response duration, progression-free survival, and survival. Time-to-event outcomes were compared using the log-rank test. Other statistical analyses were performed using the χ^2 test or Fisher's exact test, and $P < .05$ was considered to indicate statistical significance. The primary end point was the response rate, which determined the sample size. We chose a 40% response rate as a desirable target level and a 20% response rate as uninteresting in the sensitive group, with a power in excess of 80% and less than 2.5% type I error. For the refractory group, the sample size was planned using an adequate power to demonstrate that the overall response rate was greater than 5%. If the true overall response rate were assumed to be 25%, a sample size of 16 assessable patients would have a power of 80% based on a 5% α level (one-sided test) and an exact binomial distribution.

RESULTS

Between June 2003 and December 2004, 60 patients were enrolled onto this multicenter trial. Sixteen and 44 patients in the refractory and sensitive groups were eligible for the study, and assessable for toxicity, response, and survival. The characteristics of the 60 patients