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Fractionated Administration of Irinotecan and Cisplatin in Japanese Patients With Extensive-Stage–Disease Small-Cell Lung Cancer

TO THE EDITOR: We read with great interest the recent article by Hanna et al,[1] in which they reported irinotecan and cisplatin (IP) regimen was not superior to the etoposide and cisplatin (EP) regimen for extensive-stage–disease (ED) small-cell lung cancer (SCLC), even though Noda et al[2] clearly showed the superiority of IP regimen over EP regimen. We previously fractionated the schedule of IP to obtain the synergistic effect of the two drugs and to reduce toxicities.[3] The recommended doses of irinotecan and cisplatin on days 1 and 8 were determined to be 50 mg/m^2 and 60 mg/m^2, respectively. However, the phase II study for ED SCLC was stopped early because of poor outcomes in the interim analysis.[4] Despite the small number of patients in our study, the median survival time and 1-year survival rates were similar to those reported in the study by Hanna et al (Table 1). The delivered doses of irinotecan and cisplatin in their study were 1.8 times and 0.7 times as much as those of our study, respectively (Table 1). In comparison to the study by Noda et al, we should have modified fractionated administration by escalating the dose of irinotecan and reducing that of cisplatin to improve the outcomes. However, both irinotecan and cisplatin in the Hanna et al study showed more dose intensity than that reported in the Noda et al study. Hanna et al suggested that IP might therefore be a better regimen for Japanese patients. We considered fractionated administrations of IP to be inferior to the original schedules of IP (cisplatin on day 1 and irinotecan on days 1, 8, and 15) for not only American but also Japanese patients with ED SCLC based on the findings of our study.

Another explanation for the negative results of the Hanna et al study might be due to salvage chemotherapy. More patients on the IP arm received subsequent treatment with etoposide (47.2% *v* 22.6%) whereas more patients on the EP arm received subsequent treatment with topoisomerase I inhibitors including irinotecan or topotecan (33% *v* 24.1%).[1] Noda et al did not describe the use of salvage chemotherapy, which might have affected the survival difference in both arms. Because chemotherapy with fluorouracil, leucovorin, and irinotecan (FOLFIRI), followed by fluorouracil, leucovorin, and oxaliplatin (FOLFOX), had almost the same efficacy as that with FOLFOX followed by FOLFIRI in the treatment of advanced colorectal cancer,[5] IP followed by EP might therefore have had the same efficacy as EP followed by IP in the treatment of ED SCLC. To achieve a prolonged survival, the administration of all three active cytotoxic drugs (cisplatin, irinotecan, and etoposide) during the treatment course may thus be necessary.

Table 1. Irinotecan and Cisplatin for the Treatment of Extensive-Stage–Disease Small-Cell Lung Cancer

Characteristic	Study		
	Noda et al[2]	Hanna et al[1]	Takigawa et al[4]
Age, years			
Median	63	63	61
Range	30-70	37-82	41-74
Performance status 0 or 1, %	92.2	92.3	100
Delivered dose, mg/m^2/wk			
Irinotecan	36.2	39	21.4
Cisplatin	14.3	18	25.7
Median survival, months	12.8	9.3	9.4
1-year survival rate, %	58.4	35	40
Time to progression, months	6.9	4.1	5.6

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

IN REPLY: Takigawa and colleagues consider the fractionated schedule of irinotecan and cisplatin (IP) to be inferior to the original schedule given in the study by Noda et al[1] and point to this as one possible explanation for the lack of survival advantage for the IP regimen in our study[2] published in the May 1, 2006, issue of the *Journal of Clinical Oncology*. A second point raised by these authors is that salvage chemotherapy may have affected the survival outcomes and suggest the best outcomes may be achieved with the combination of all three agents (cisplatin, etoposide, and irinotecan).

Regarding the first point, we acknowledged in our paper that the fractionated regimen of IP may be inferior to the regimen in the study by Noda et al.[1] The authors cite their own study of fractionated IP as evidence of this point.[3] However, the response rate of 80% and median time to progression of 5.6 months in their study (n = 15) was similar to that seen with the Noda IP regimen. In addition, as the authors acknowledge the dose intensity of irinotecan was 1.8 times greater with irinotecan in our study compared with theirs. The Southwest Oncology Group is completing a much larger trial in patients with extensive disease small-cell lung cancer utilizing the two arms of the Noda trial.[1] The results from this trial will provide the answer to this question of dose/schedule of IP. However, given the lack of positive phase III trials testing a number of active agents in various combinations, schedules, and dosages in extensive disease small-cell lung cancer over the last 25 years, it seems unlikely that a change in schedule of IP which provides less dose intensity (as does the original schedule of IP compared with our regimen) will positively affect survival outcomes.

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A phase I and pharmacological study of amrubicin and topotecan in patients of small-cell lung cancer with relapsed or extensive-disease small-cell lung cancer

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Summary Cisplatin-based chemotherapy is considered to be a standard treatment in patients with relapsed or extensive-disease (ED) small-cell lung cancer (SCLC), the survival benefit remains modest. Relapsed or ED-SCLC patients were enrolled. Topotecan and amrubicin were administered on Days 1–5 and on Days 3–5, respectively. Nine patients received a total of 24 cycles. Since all three patients experienced dose-limiting toxicity (grade 4 neutropenia lasting for more than 4 days, grade 3 febrile neutropenia, and grade 4 thrombocytopenia) at the third dose level (topotecan: 0.75 mg/m^2, amrubicin 40 mg/m^2), the maximum tolerated dose was determined to be this dose level. Objective response was observed in six patients (67%). The maximum concentration (C_{max}) and area under the plasma concentration–time curve (AUC) of amrubicin increased in a dose-dependent manner. Amrubicin did not influence the pharmacokinetics of topotecan. The C_{max} and AUC of amrubicin were correlated with the duration of grade 4 neutropenia. The mean C_{max} of topotecan on day 2 in responders (22.9 ± 3.6) was significantly higher than that in non-responders (10.9 ± 0.4). This phase I study showed the safety and activity of two-drug combination of amrubicin and topotecan in patients with relapsed or ED-SCLC.

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1. Introduction

Recently, therapy with a cisplatin (CDDP)-based two-drug combination has been used as the standard treatment for small-cell lung cancer (SCLC) cases with extensive-disease (ED). In particular, the combination of irinotecan (CPT-11) and CDDP has been reported to be highly effective in previously untreated patients with ED-SCLC [1]. However, since the majority of responders showed early relapse, and salvage chemotherapy for SCLC usually yields disappointing results, the long-time survival rate was extremely low [2–5]. Accordingly, in order to achieve better treatment results for SCLC, new effective combination regimens need to be sought for patients with relapsed or refractory SCLC after standard chemotherapy. Recently, several new agents with novel mechanisms of actions have been developed and been shown to be highly effective for the treatment of SCLC [6].

Amrubicin (AMR), a novel and entirely synthetic anthracycline, inhibits DNA topoisomerase II activity. It has been shown to be active against previously untreated SCLC, with an overall response rate and median survival time (MST) of 78.8% and 11.0 months, respectively [7].

Topotecan (TOP), a unique semi-synthetic water-soluble analog of camptothecin, exhibits inhibitory activity against DNA topoisomerase I, and has been shown to have favourable anti-tumour activity against SCLC, with a response rate of 39% and MST of 9.0 months [8].

DNA topoisomerases I and II are functionally correlated and act in concert. Both enzymes are believed to be essential for the maintenance of cell viability. Therefore, combined use of agents targeted against the DNA topoisomerases I and II may be expected to completely inhibit both DNA and RNA synthesis and exert synergistic cytotoxicity [9–11]. There have been some reports of the effectiveness of such a combination of drugs, namely, irinotecan (CPT-11) and etoposide (VP-16), in patients with SCLC [12].

Based on these results, we conducted a phase I trial of the two-drug combination chemotherapeutic regimen of AMR and TOP in patients with relapsed or ED-SCLC. The primary objective of this trial was to determine the maximum tolerated dose (MTD) of the two-drug regimen. The secondary objectives were to investigate the anti-tumour activity of the regimen and influence of the administration sequence of the two drugs on the pharmacokinetics and clinical toxicity of the combination regimen.

2. Materials and methods

2.1. Eligibility criteria

Patients were recruited based on the following eligibility criteria: pathologically proven SCLC; relapsed disease or ED-SCLC; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0, 1 or 2; age $\leq$75 years; presence of evaluable lesion; no chemotherapy within 4 weeks prior to study entry; adequate haematological (WBC count $\geq$3000/μL, neutrophil count $\geq$1500/μL, haemoglobin level $\geq$9.5 g/dL, platelet count $\geq 15 \times 10^4/\mu$L), renal (serum creatinine $\leq$ 1.5 mg/dL), hepatic (total bilirubin $\leq$ 1.5 mg/dL, serum transaminases $\leq$2.5$\times$ upper limit of normal range) and pulmonary function ($PaO_2 \geq 60$ Torr) reserves; receipt of written informed consent. Patients with symptomatic brain metastasis or evidence of preexisting interstitial pulmonary disease on the chest radiograph were excluded from the study. Pretreatment evaluations included a complete history, physical examination, laboratory tests, chest radiography, electrocardiography, computed tomography (CT) of the chest and abdomen, magnetic resonance imaging (MRI) of the brain, and a radionuclide bone scan. Staging was conducted according to the tumour, node, metastasis system [13]. The protocol was approved by the institutional review board of the NHO Minami-Okayama Hospital and Okayama University Medical School.

2.2. Treatment scheme

TOP, diluted in 100 mL of physiological saline, was administered by intravenous infusion over 1 h on days 1–5. AMR, diluted in 20 mL of physiological saline, was administered as a bolus intravenous injection over 5 min on days 3–5, after completion of the TOP infusion. Each patient was premedicated with i.v. dexamethasone (8 mg) and granisetron (3 mg). The starting doses of TOP and AMR were 0.75 and 30 mg/m^2, respectively, which were 60–70% of the recommended doses in previous phase II monotherapy studies [8,14–16]. The following five dose escalations of TOP/AMR (mg/m^2) were planned: 0.75/30, 0.75/35, 0.75/40, 1.0/40 and 1.0/45.

The treatment was repeated every 4 weeks at the same dose levels up to four cycles, unless disease progression or unacceptable toxicity was observed, or the patient refused further treatment. Initiation of the next cycle of chemotherapy was delayed until recovery of the WBC count to $\geq$3000/μL, neutrophil count to $\geq$1500/μL, platelet count to $\geq 15 \times 10^4/\mu$L, and resolution of non-haematologic toxicities to $\leq$grade 1. After completion or discontinuation of this regimen, patients were permitted to receive standard chemotherapy for SCLC.

2.3. Assessment of toxicity and dose escalation

Toxicity was graded according to the National Cancer Institute-Common Toxicity Criteria ver 2.0 [17]. Dose-limiting toxicity (DLT) was defined as development of at least one of the following adverse events: any non-haematologic toxicities $\geq$grade 3, except for alopecia, nausea, vomiting and general malaise; platelet count $\leq 2 \times 10^4/\mu$L; grade 4 leukopenia; persistence of grade 4 neutropenia for more than 4 days; grade 3 or more severe neutropenia with fever $\geq$38 °C or evidence of infection; failure to recover sufficiently from toxicities by Day 29, before beginning the next cycle of treatment.

Initially, three patients were enrolled at each dose level. If fewer than two patients experienced DLT, the next group of patients was treated at the next higher dose level. If all three patients developed the DLT, the dose level was determined to be the MTD. The recommended dose was also defined as one below the MTD. If two patients experienced the DLT, six patients in total were administered the same dose level. If half or more of these six patients developed DLT, the dose was determined to be the MTD. Dose escalation above the starting dose in individual patients was

not allowed. If grade 4 leukopenia, grade 4 neutropenia, or febrile neutropenia was noted, the use of granulocyte colony-stimulating factor (G-CSF) was permitted.

2.4. Assessment of antitumour activity

The standard response evaluation criteria in solid tumors was used to evaluate the responses [18]. Complete and partial response (PR) were confirmed by two observations not less than 4 weeks apart.

2.5. Pharmacokinetic analysis

Blood samples for pharmacokinetic analysis were obtained during the second and third days of the first cycle, from an indwelling venous catheter placed in the arm contralateral to that used for the drug infusion. Five milliliters of blood were collected in heparinised tubes before the drug administration, at the end of the TOP infusion, and 0.5, 3, 8 and 23 h after the end of the TOP infusion on both Days 2 and 3 in the first cycle. After centrifugation, the plasma specimens were stored at −80 °C until the assays. The plasma concentrations of AMR, amrubicinol (13-OH-AMR: active form of AMR) and TOP were measured by high-performance liquid chromatography (HPLC). The area under the plasma concentration–time curve (AUC) was calculated using WINNONLIN Standard Edition, Version 1.5. Differences in the pharmacokinetic parameters among three dose levels in the first cycle were evaluated by the Kruskal–Wallis test, and those between Days 2 and 3 in the first cycle were evaluated by Mann–Whitney's *U*-test. The correlations between the pharmacokinetic parameters and the clinical toxicities or responses were assessed with Spearman's rank test. Statistical analyses were performed using the STATVIEW 5.0 program (Brainpower, Calabasas, CA). A *p*-value of less than 0.05 was considered to denote statistical significance.

3. Results

3.1. Patients' characteristics

Nine patients with relapsed or ED-SCLC were enrolled between April and November 2003. There were eight men and one woman, with a median age of 62 years (range, 51–75 years). All patients had a good performance status (PS 0 in five patients and PS 1 in four patients). Five patients (56%) had received prior chemotherapy (CDDP + VP-16 in three, CDDP + CPT-11 in one, and carboplatin + VP-16 in one). Three patients had sensitive disease and two had refractory disease.

A total of 24 chemotherapy cycles were administered. Three patients (33%) received only one cycle of chemotherapy, because of unacceptable toxicity (two patients) or the patient's refusal to undergo further treatment (one patient). At the first dose level (TOP 0.75 mg/m^2, AMR 30 mg/m^2), one patient developed DLT (grade 3: diarrhoea, stomatitis and febrile neutropenia, grade 4: leukopenia, neutropenia lasting for more than 4 days and thrombocytopenia). At the second dose level (TOP 0.75 mg/m^2, AMR 35 mg/m^2), one patient developed DLT (grade 4 neutropenia lasting for more than 4 days). At the third dose level (TOP 0.75 mg/m^2, AMR 40 mg/m^2), all three patients experienced DLT (grade 4 neutropenia lasting for more than 4 days in one, grade 4 neutropenia lasting for more than 4 days and grade 3 febrile neutropenia in one patient each, and grade 4 thrombocytopenia in one). Therefore, the third dose level was deemed to be MTD, and the recommended doses for the phase II study were the second dose levels, that is, 0.75 mg/m^2 for TOP, and 35 mg/m^2 for AMR.

Table 1 Grade 2 or more severe haematological toxicity (all courses)

Toxicity	Grade	Dose level		
		1	2	3
No. of treated patients		3	3	3
No. of courses evaluated		7	9	8
No. of courses in which toxicity was encountered (%)				
Leukopenia	2	0	1 (11%)	1 (13%)
	3	6 (86%)	8 (89%)	3 (38%)
	4	1 (14%)	0	4 (50%)
Neutropenia	2	1 (14%)	0	2 (25%)
	3	2 (29%)	3 (33%)	0
	4	4 (57%)	6 (67%)	6 (75%)
Thrombocytopenia	2	1 (14%)	4 (44%)	0
	3	1 (14%)	0	5 (63%)
	4	1 (14%)	0	0
Anaemia	2	1 (14%)	5 (56%)	3 (38%)
	3	1 (14%)	2 (22%)	2 (25%)
	4	2 (29%)	0	1 (13%)

3.2. Haematological toxicity

The main toxicity of this drug combination was myelosuppression. Analysis of the toxicity during all courses of chemotherapy is shown in Table 1. Grade 3 or 4 leukopenia was observed during all the seven courses (100%) at the first dose level, eight courses (89%) at the second dose level, and seven courses (88%) at the third dose level. Similarly, grade 3 or 4 neutropenia was also frequently observed, necessitating G-CSF administration in eight patients. Grade 3 or 4 thrombocytopenia was observed less frequently at the first and second dose level, however it was observed during five courses (63%) at the third dose level, with two patients requiring platelet transfusion. Although grade 3 or 4 anaemia was observed less frequently, three patients required red blood cell transfusion.

3.3. Non-haematological toxicity

The non-haematological toxicities observed are summarised in Table 2. Febrile neutropenia occurred during one course (14%) at the first dose level, two courses (22%) at the second dose level, and four courses (50%) at the third dose level, however, it was reversible in all cases with only appropriate supportive care. Other toxicities, including diarrhoea, were mild, and did not require any intensive management.

There seemed to be different severity in toxicity profiles in patients with or without prior chemotherapy; grade 4 neutropenia and leucopenia were observed in 5 (38%) of 13 courses versus none of 11 courses in previously treated and untreated patients, respectively. Additionally, febrile neutropenia occurred in only patients with prior chemotherapy (7 [54%] of 13 courses versus none of 11 courses, respectively). However, in our study, pretreated patients tended to be incidentally accrued at higher dose level, which might be rather contributed to the difference in severity of toxicity profiles than prior chemotherapy itself was.

3.4. Antitumour activity

All patients were assessable for response. Although none of the cases showed complete response, six patients (67%),

Table 2 Grade 2 or more severe non-haematologic toxicity (all courses)

Toxicity	Grade[a]	Dose level		
		1	2	3
No. of treated patients		3	3	3
No. of courses evaluated		7	9	8
No. of courses in which toxicity was encountered (%)				
Febrile neutropenia	3	1 (14%)	2 (22%)	4 (50%)
Nausea/vomiting	2	0	1 (11%)	0
	3	0	0	0
Hepatotoxicity	2	1 (14%)	0	0
	3	0	0	0
Infection	2	0	0	0
	3	0	1 (11%)	0
Diarrhoea	2	0	1 (11%)	0
	3	1 (14%)	0	0

[a]No grade 4 or more severe toxicities were observed.

Table 3 Pharmacokinetic parameters of the drugs at dose levels 1–3

		Level 1 (AMR 30 mg/m^2) [number of points: 3]	Level 2 (AMR 35 mg/m^2) [number of points: 3]	Level 3 (AMR 40 mg/m^2) [number of points: 3]	p
AMR	C_{max} (ng/mL)	319.4 ± 109.5	401.6 ± 76.1	447.5 ± 33.5	0.49
	AUC (ng h/mL)	1195.6 ± 445.5	1615.1 ± 194.6	1849.8 ± 90.2	0.58
13-OH-AMR	C_{max} (ng/mL)	23.2 ± 13.3	28.9 ± 2.5	28.3 ± 2.5	0.73
	AUC (ng h/mL)	196.2 ± 169.7	191.2 ± 95.3	299.4 ± 88.2	0.67
TOP (day 2)	C_{max} (ng/mL)	20.3 ± 2.9	21.6 ± 7.9	18.8 ± 7.5	0.73
	AUC (ng h/mL)	64.2 ± 5.1	54.3 ± 15.7	45.1 ± 5.9	0.25
TOP (day 3)	C_{max} (ng/mL)	22.1 ± 1.7	15.0 ± 1.1	16.8 ± 1.7	0.09
	AUC (ng h/mL)	71.4 ± 6.7	53.2 ± 6.2	56.5 ± 1.9	0.19

Each data represents the mean values and standard errors. *Abbreviations*: AMR, amrubicin; TOP, topotecan; C_{max}, maximum concentration; AUC, area under the plasma concentration–time curve.

Table 4 Pharmacokinetic parameters of topotecan on days 2 and 3

	Day 2 (topotecan alone) [number of points: 9]	Day 3 (topotecan combined with amrubicin) [number of points: 9]	p
Parameters			
T_{max} (h)	0.5	0.5	
C_{max} (ng/mL)	20.2±3.3	18.0±1.3	0.83
AUC (ng h/mL)	54.5±5.8	60.4±3.9	0.23

Each data represents the mean values and standard errors. Abbreviations: C_{max}, maximum concentration; AUC, area under the plasma concentration–time curve.

including one receiving only the first dose level, showed PR. It is worthy of note that 4 out of the 5 (80%) relapsed patients showed PR, although only 2 out of 4 (50%) chemonaïve patients showed PR. The median time to progression was 4.0 (95% CI: 0.8–6.8) months.

3.5. Pharmacokinetic and pharmacodynamic analysis

Pharmacokinetic parameters were determined in samples obtained on the second and third days of the first cycle in all nine patients. The maximum concentration (C_{max}) and AUC of AMR increased in a dose-dependent manner, although statistical significance was not reached (Table 3). The C_{max} and AUC of TOP were almost comparable among the first three dose levels, suggesting that the AMR dose did not influence the pharmacokinetics of TOP (Table 3). The C_{max} and AUC of

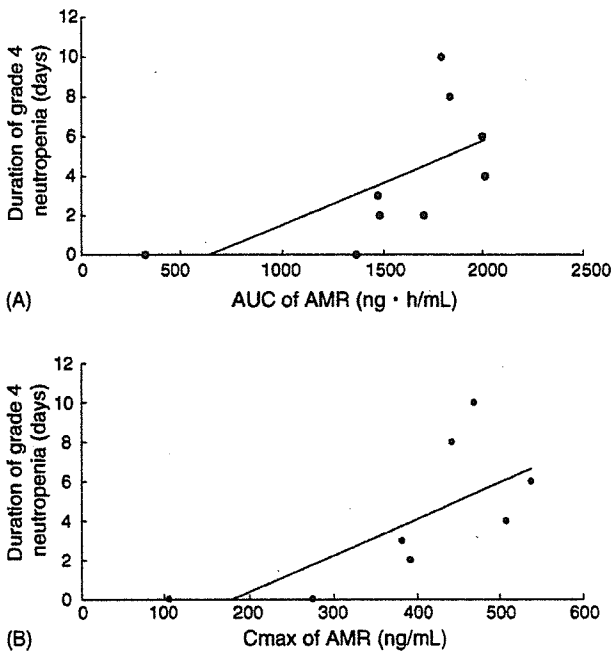

Fig. 1 (A) The correlation between the area under the plasma concentration–time curve (AUC) of AMR (amrubicin) on day 2 and the duration of grade 4 neutropenia in the first cycle (Spearman rank test, $p=0.0288$), and (B) the correlation between the maximum concentration (C_{max}) of AMR on day 2 and the duration of grade 4 neutropenia in the first cycle (Spearman rank test, $p=0.0225$).

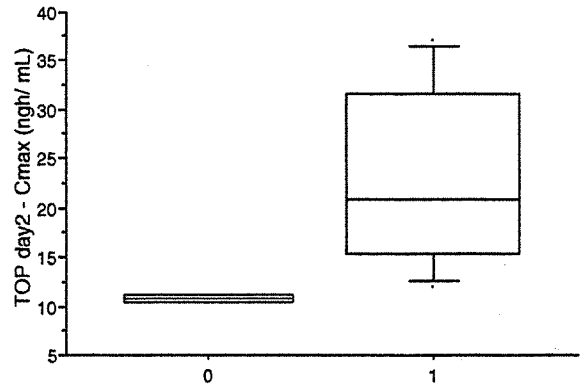

Fig. 2 Correlation between the maximum concentration (C_{max}) of topotecan on day 2 and objective tumour response in the first cycle. "0" denotes stable disease and progressive disease and "1" denotes partial response. The mean C_{max} of seven responders and two non-responders were 22.9±3.6 and 10.9±0.4, respectively (Mann–Whitney's U-test, $p=0.0404$).

13-OH-AMR were not significantly different even with dose escalation of AMR. 13-OH-AMR was not detectable in any of the samples collected from the first patient and two of the samples collected from the second patient at the first dose level, in three samples collected from the two patients at the second dose level, and in one sample collected from the patients at the third dose level, although AMR was detectable in all of these samples. However, the serum concentrations of 13-OH-AMR were higher than 20 ng/mL (minimum detectable value) in all the other patients. We also evaluated differences in the pharmacokinetic parameters of TOP between Day 2 (TOP alone) and Day 3 (TOP plus AMR), in order to investigate the effect of concurrent administration of AMR on the pharmacokinetics of TOP. As listed in Table 4, there were no significant differences. In the correlation of toxicity profiles with the pharmacokinetic parameters, the AUC and C_{max} of AMR were correlated with the duration of grade 4 neutropenia ($p=0.0288$ and 0.0225, respectively; Fig. 1A and B). In addition, the mean C_{max} of TOP on Day 2 in 7 responders (22.9±3.6) was significantly higher than that in 2 non-responders (10.9±0.4, $p=0.0404$; Fig. 2).

4. Discussion

Although the combined use of DNA topoisomerase I and II inhibitors is theoretically attractive, preclinical studies have demonstrated mixed results [19,20–23]. There have been

some reports of the clinical evaluation of such a drug combination, namely irinotecan (CPT-11) and etoposide (VP-16) [12,24,25]. Although Masuda et al. [12] concluded that the combination regimen of CPT-11 and VP-16 was effective against refractory or relapsed SCLC, no further studies have been reported. In this study, we investigated the feasibility and effectiveness of the combination chemotherapeutic regimen of AMR (DNA topoisomerase II inhibitor) and TOP (DNA topoisomerase I inhibitor) in patients with relapsed or ED-SCLC.

The rationale for combining DNA topoisomerase I and II inhibitors is that such a combination of drugs would yield greater inhibition of the DNA topoisomerase activity resulting in more potent cytotoxicity, because each topoisomerase enzyme has some compensatory activity in the event of deficiency of the other. It has been reported that the cytotoxicity of such a drug combination increases when the drugs are administered sequentially [22,23]. Kim stressed the importance of the administration sequence in a preclinical study, and showed that administration of CPT-11 (topoisomerase I inhibitor) before doxorubicin (topoisomerase II inhibitor) resulted in a synergistic effect against human tumour xenografts in nude mice [23]. However, Masuda reported that administration of VP-16 (topoisomerase II inhibitor) before CPT-11 was also effective in a clinical study [12,25]. In this study, we administered TOP before AMR, and obtained favourable results. Therefore, clinically, the sequence of administration of the two drugs may not be very important.

The present study demonstrated that treatment with the drug combination of TOP and AMR is feasible in patients with relapsed or ED-SCLC. Negoro, et al. [14] reported the results of a phase I study of AMR monotherapy, with daily administration of the drug for three consecutive days. The MTD was 50 mg/m^2/day (150 mg/m^2/course), and the DLTs were leukopenia, neutropenia, thrombocytopenia and gastrointestinal toxicities. On the other hand, the MTD of TOP during 5 days' administration was estimated to be 1.5–2.0 mg/m^2/day, and the DLTs were reversible leukopenia and neutropenia [15,16]. Subsequently, the clinical effectiveness of a combination of DNA topoisomerase I and II inhibitors, that is, CPT-11 and VP-16, was reported by Karato [24] and Masuda [25]. In Karato's study [24], both drugs were administered on Days 1–3 with G-CSF support. The MTDs of VP-16/CPT-11 were 60/80 or 80/60 mg/m^2, and the DLTs were weight loss and diarrhoea. In Masuda's study [25], CPT-11 was administered on Days 1, 8 and 15, and VP-16 was given on Days 1–3 with G-SCF support. The MTD of CPT-11 was 90 mg/m^2 and that of VP-16 was 80 mg/m^2. The DLTs were diarrhoea and leukopenia. During treatment with the chemotherapeutic combination of TOP and AMR in our study, we determined the MTD of TOP and AMR to be 0.75 mg/m^2 and 50 mg/m^2, respectively. The DLT was almost limited to haematological toxicities and seemed severe, however, all these toxicities were reversible, and we finally considered the phase II dose to be the level 2 dose according to the initial definition for the recommended dose, although further investigation is needed to confirm its safety profiles in the following studies using larger cohorts.

In this study, the C_{max} and AUC of AMR increased in a dose-dependent manner, and statistical significance was not reached. However, the corresponding values of 13-OH-AMR varied markedly among the patients, perhaps attributable partly to our small patient population. However, Ohe et al. also demonstrated similar results with respect to 13-OH-AMR in red blood cells in a phase I/II trial of AMR and CDDP in 45 chemo-naïve patients with ED-SCLC [26]. Negoro, et al. [14] also documented that the plasma concentrations of 13-OH-AMR were very low as compared to those of AMR. Thus, it may be difficult to construct a limited sampling model for estimating the AUC of 13-OH-AMR in either single-agent therapy or combination therapy. The C_{max} and AUC of TOP were not significantly different among the first three dose levels, or between Days 2 and 3, which indicates that AMR did not influence the pharmacokinetics of TOP.

In the pharmacodynamic analysis, we demonstrated that the Cmax and AUC of AMR were correlated with the duration of grade 4 neutropenia. In addition, the mean C_{max} of TOP on Day 2 in seven responders was significantly higher than that in two non-responders. Concerning the relationship between the antitumour effect and pharmacokinetics of AMR, Noguchi et al. reported that the AUC of intracellular 13-OH-AMR was related to the anti-tumour effect of the drug [27]. However, these relationships were not observed in our study. It remains unknown why the Cmax of TOP on the previous day used together with AMR was associated with an objective response. Further investigation is warranted to confirm the role of pharmacokinetic and pharmacodynamic monitoring during treatment with the combination regimen of AMR and TOP.

Using CPT-11 and VP-16, a combination of DNA topoisomerase I and II inhibitors, Masuda et al. [12] reported favourable outcomes in cases of refractory or relapsed SCLC. Among the 24 assessable patients, complete response was observed in three (13%), while 14 (58%) patients showed a PR, with an overall response rate of 71%. The response rate was particularly high (80%) in patients with relapsed SCLC. In this study also, the PR rate in relapsed cancer patients was extremely high (80%). Kubota et al. [28] reported a high response rate of 88% to the CODE regimen in 17 relapsed SCLC patients, which was associated with an encouraging survival rate (MST: 245 days). Therefore, we may expect survival benefit with the use of this combination, and this should be confirmed in future studies.

5. Conclusion

In conclusion, this phase I study showed both the feasibility and effectiveness of the two-drug combination of TOP and AMR in patients with relapsed or ED-SCLC. Since this combination seems to be particularly effective for relapsed SCLC, a phase II trial of this drug regimen in this subset of patients (relapsed SCLC) is warranted.

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The combination effect of amrubicin with cisplatin or irinotecan for small-cell lung cancer cells

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Abstract. The single agent of amrubicin is active in untreated small-cell lung cancer (SCLC). Cytotoxicity of amrubicinol, the active form of amrubicin, was evaluated in a parent SCLC cell line (SBC-3); an active metabolite of irinotecan, 7-ethyl-10-hydroxy-camptothecin (SN-38)-resistant subline (SBC-3/SN-38); and cisplatin-resistant subline (SBC-3/CDDP) using AlamarBlue assay. Interaction of the combined drugs was evaluated by median-effect plot analysis, and the fraction of apoptotic cells was determined using flow cytometry. SBC-3/SN-38 was 34-fold more resistant to SN-38 and SBC-3/CDDP was 7.2-fold more resistant to cisplatin than parental SBC-3. However, these resistant sublines retained sensitivity to amrubicinol (1.8- and 1.7-fold, respectively). Simultaneous exposure of SBC-3/SN-38 cells to amrubicinol and cisplatin showed a synergistic effect. Simultaneous exposure of SBC-3/CDDP cells to amrubicinol and SN-38 displayed synergistic or additive effects. The two-drug combination produced an increase of apoptotic cells compared to each single agent alone in both resistant cells. These findings suggest that amrubicin alone and in combination with cisplatin or irinotecan is effective against SCLC refractory to irinotecan and/or cisplatin.

Introduction

More than 80% of patients with small-cell lung cancer (SCLC) receiving chemotherapy achieve an objective response; however, most responders eventually relapse because of drug resistance (1). Since a phase III study in patients with extensive disease (ED)-SCLC demonstrated that a combination regimen of cisplatin and irinotecan yielded a highly significant improvement in survival over a standard regimen consisting of cisplatin and etoposide (2), the combination may be considered the current standard treatment for ED-SCLC. However, the median survival time and 2-year survival rate were only 12.8 months and 19.5%, respectively (2). The development of irinotecan or cisplatin resistance in tumor cells is assumed to play a major role in these unsatisfactory results.

Amrubicin is a totally synthetic 9-aminoanthracyclin (3). Amrubicinol, its converted active form, has 10 to 100 times higher activity than amrubicin in cytotoxicity by inhibiting topoisomerase II (4,5). Antitumor activity of amrubicin was superior to that of the mother compound, adriamycin in human tumor xenografts (6). In addition, amrubicin had less toxicity, including cardiotoxicity, than adriamycin, in experimental animal models (7,8). Amrubicin was highly active (response rate, 78.8%; median survival time, 11.3 months) and well tolerated in a phase II study in untreated patients with ED-SCLC (9). The objectives of this study were to evaluate the antitumor activity of amrubicin for SCLC cells, especially for irinotecan- or cisplatin-resistant cells, and the combination effect of amrubicin with commonly used anticancer drugs against SCLC.

Materials and methods

Chemicals and reagents. Drugs in this study were provided by the following sources: amrubicin (SM5887) and amrubicinol (SM5887-13-OH) from Sumitomo Pharmaceuticals Co., Ltd., Osaka, Japan; irinotecan and 7-ethyl-10-hydroxycamptothecin (SN-38) from Yakult Honsha, Tokyo, Japan; etoposide and paclitaxel from Bristol-Myers Squibb, Tokyo, Japan; and cisplatin from Nippon Kayaku Kogyo Co., Ltd., Tokyo, Japan. Amrubicin, irinotecan and cisplatin were dissolved in 0.9% saline, and amrubicinol was dissolved in distilled

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water. SN-38, etoposide, and paclitaxel were dissolved in dimethylsulfoxide. Drug solutions were stored at -20°C. AlamarBlue (UK Serotec Ltd., Oxford) was purchased from Dainippon Pharmaceutical Co. Ltd, Osaka, Japan.

Cell culture. The SBC-3 parent cell line was established from a bone marrow aspirate of a previously untreated patient with SCLC (10). The growth medium (RPMI-FBS) was RPMI-1640 supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA). The SN-38-resistant subline (SBC-3/SN-38) (11) and cisplatin-resistant subline (SBC-3/CDDP) (12) were established by continuous exposure of the SBC-3 cells to increasing concentrations of SN-38 and cisplatin, respectively.

Assay of drug sensitivity. Drug sensitivity was determined using AlamarBlue assay (13). Briefly, 50 μl of RPMI-FBS containing serial concentrations of each chemotherapeutic agent was prepared in 96-well flat-bottomed microplates (Coster 3596; Corning Inc., Corning, NY, USA). The 50 μl of RPMI-FBS containing 500 cells for SBC-3, 1500 cells for SBC-3/SN-38 and 2000 cells for SBC-3/CDDP was then added to each well. Cells were incubated at 37°C for 96 h in a highly humidified incubator with 5% CO_2 and 95% air, and then 10 μl of AlamarBlue was added to each well. After incubation at 37°C for 5 h, the fluorescence of each well was measured using Fluoroskan Ascent (Labsystems Inc., Franklin, MA, USA) with 544 nm excitation and 590 nm emission. Fluorescence of a well without chemotherapeutic agents was used as the control, and a well containing only RPMI-FBS and AlamarBlue was used to determine the background. The percentage of surviving cells was calculated using the formula: [(mean fluorescence in 4 test wells - fluorescence in background wells)/(mean fluorescence in control wells - fluorescence in background wells)] x100. The drug concentration required to inhibit growth of tumor cells by 50% (IC_{50}) was determined by plotting the logarithm of drug concentration versus the percentage of surviving cells.

Table I. Drug sensitivity in the SBC-3 parent line, SN-38-resistant subline (SBC-3/SN-38), and cisplatin-resistant subline (SBC-3/CDDP).

	IC_{50} value (nM)		
	SBC-3	SBC-3/SN-38	SBC-3/CDDP
SN-38	4.1±1.5	139±16	13±4.5
R.R.		34	3.2
Cisplatin	345±39	120±15	2480±120
R.R.		0.35	7.2
Amrubicinol	33±16	60±26	57±20
R.R		1.8	1.7

IC_{50}, 50% inhibitory concentration; SD, standard deviation; R.R, relative resistance value (IC_{50} value of resistant cells/IC_{50} value of SBC-3 cells). Data are expressed as mean ± SD.

Determinations were carried out in quadruplicate for each experiment, and results were confirmed by 3 or more separate experiments. Relative resistance was calculated by dividing the IC_{50} value of resistant subline cells by the IC_{50} of SBC-3 cells.

Design for drug combination. The constant-ratio design for the combination assay is highly recommended as it allows the most efficient data analysis (14). After simultaneous exposure of the cells to two drugs for 96 h, growth inhibition was determined using AlamarBlue assay. Sequential exposure of two drugs was performed as follows. After exposure to the first drug for 24 h, cells were twice washed in drug-free medium, and the second drug was then added to the 96-well microplates for 24 h. At the end of exposure, the cells were washed in drug-free medium, re-incubated in drug-free medium for 48 h, and proliferation was measured with AlamarBlue. Experiments were repeated 3 times.

Table II. Combination effect of amrubicinol and other agents.

Cell line	Drugs	Combination index (mean ± SD)	
		IC_{70}	IC_{90}
SBC-3	AMR-OH + SN-38	1.2±0.1	1.0±0.02
	AMR-OH + CDDP	0.82±0.05	0.35±0.17
	AMR-OH + PTX	1.3±0.26	2.4±0.52
	AMR-OH + ETP	1.1±0.02	0.85±0.21
	AMR-OH → SN-38	1.0±0.02	1.1±0.25
	SN-38 → AMR-OH	1.5±0.32	2.2±0.17
	AMR-OH → CDDP	0.86±0.15	0.93±0.32
	CDDP → AMR-OH	0.93±0.12	1.0±0.06
SBC-3/CDDP	AMR-OH + SN-38	0.76±0.21	1.0±0.35
SBC-3/SN-38	AMR-OH + CDDP	0.99±0.17	0.89±0.24

AMR-OH, amrubicinol; CDDP, cisplatin; PTX, paclitaxel; ETP, etoposide.

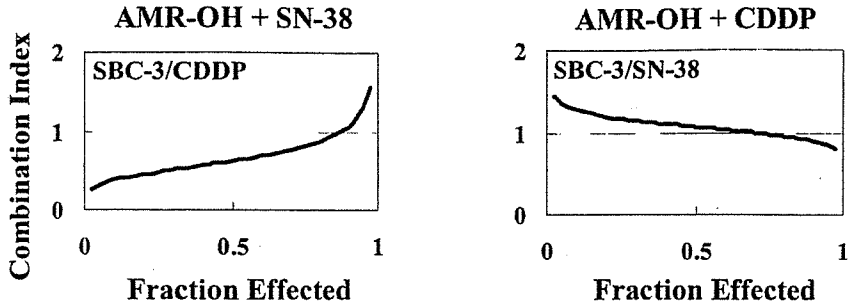

Figure 1. Combination index and surviving fraction of SBC-3/CDDP cells treated with amrubicinol (AMR-OH) in combination with SN-38 simultaneously for 96 h (left). Combination index and surviving fraction of SBC-3/SN-38 cells treated with AMR-OH in combination with cisplatin (CDDP) simultaneously for 96 h (right).

Median-effect principle for dose-effect analysis. The multiple drug effect analysis of Chou and Talaly, based on the median-effect principle, was used to calculate the combined drug effect (15). This method involved plotting dose-effect curves for each agent and its combination with other agents by using the median-effect equation: $fa/fu = (D/Dm)^m$ (equation 1).

In equation 1, D is the dose, Dm is the required dose for 50% inhibition of cell growth, *fa* is the fraction affected by dose D (e.g. 0.9 if cell growth is inhibited by 90%), *fu* is the unaffected fraction (therefore, fa = 1-fu), and *m* is a coefficient of the sigmoidicity of the dose-effect curve; $m=1$, $m>1$, and $m<1$ indicate hyperbolic, sigmoidal, and negative sigmoidal dose-effect curves, respectively, for an inhibitory drug. Thus, both potency (Dm) and shape (*m*) were taken into account as parameters in this method. Equation 2 was rearranged from equation 1 as follows: $D = Dm[fa/(1-fa)]^{1/m}$ (equation 2).

The Dm and m values were easily determined by the median-effect plot; x= log (D) versus y= log (*fa/fu*) was based on the logarithmic form of equation 1. In the median-effect plot, *m* was slope and log (Dm) was the x-intercept. Conformity of data to the median-effect principle could be readily manifested by the linear coefficient (r) of the median-effect plot. To obtain a reasonable *m* and *r*, non-linear points, usually at the lowest or the highest concentrations, were excluded. The 5 to 9 concentrations on a linear line were employed in this analysis. Computer programs based on the median-effect plot parameters and combination index equation have been used for data analysis in the present study (16).

Combination index for determining synergism and antagonism. The combination index (CI) isobologram equation was used for data analysis of the two-drug combination: CI = (D)A/(Dx)A + (D)B/(Dx)B (equation 3).

CI<1, CI=1, and CI> 1 indicate synergism, additive effect, and antagonism, respectively. Equation 3 dictates that drug A, i.e. (D)B in the numerators inhibit x% when drugs A and B are combined. (Dx)A and (Dx)B in denominators of equation 3 indicate doses of drug A and drug B alone, respectively, that also inhibit x%. Dx can be readily calculated from equation 2, where D is designated for x% inhibition. When equation 3 equals 1 (i.e. CI=1), it represents the classic isobologram equation. CI at the inhibitory concentration of 70% (IC_{70}) and 90% (IC_{90}) levels was used for determining synergism, additive effect, or antagonism.

Flow cytometry. Flow cytometry for cell cycle traverse perturbations was carried out after staining with propidium iodide using CycleTest Plus DNA Reagent kit (Becton-Dickinson Immunocytometry Systems, San Jose, CA, USA). Drug concentration was based on the IC_{50} value of a single drug. After 96 h simultaneous exposure to single drug or combined drugs, cells were stained according to the instruction manual. For sequential schedules, after 24 h of exposure to the first drug, cells were twice washed in drug-free medium, and the second drug was then added to cells for 24 h. At the end of exposure, cells were stained with propidium iodide. Flow cytometric analysis was performed on a FACSCalibur (Becton-Dickinson Immunocytometry Systems). Data were analyzed according to ModFit LT software (Verity Software House Inc, Topsham, ME, USA).

Results

Cytotoxicity of amrubicinol and other drugs. Values (mean ± standard deviation) for IC_{50} and relative resistance of SN-38, cisplatin, and amrubicinol for SBC-3, SBC-3/SN-38, and SBC-3/CDDP cells are shown in Table I. Although SBC-3/SN-38 was 34-fold more resistant to SN-38 and SBC-3/CDDP was 7.2-fold more resistant to cisplatin than the parental SBC-3, they retained sensitivity to amrubicinol with relative resistance values of 1.8 and 1.7, respectively. IC_{50} values of other drugs for SBC-3 cells were: amrubicin, 862±46 nM; irinotecan, 195±10.2 nM; etoposide, 270±170 nM; and paclitaxel, 0.55±0.25 nM.

Combination effect of amrubicinol with other drugs for SBC-3. To equalize the contribution of each drug, the ratio of IC_{50} value for each drug was used as the concentration ratio for the combination (14). Thus, concentration ratios of amrubicinol, SN-38, cisplatin, paclitaxel, and etoposide were designed to be relative ratios of 100: 10: 1000: 1:1000, respectively. CI values for SBC-3 cells treated with amrubicinol after 96 h simultaneous exposure to SN-38, paclitaxel, cisplatin or etoposide are shown in Table II. Amrubicinol and cisplatin showed a synergistic effect, however, amrubicinol and paclitaxel exerted an antagonistic

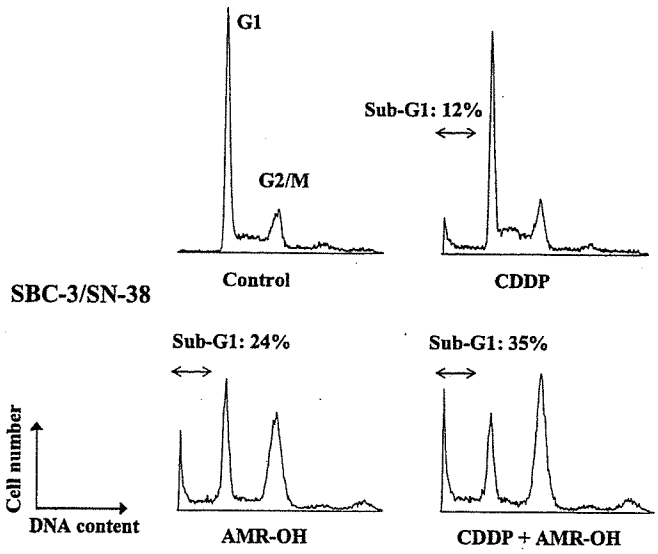

Figure 2. Effect of cisplatin (CDDP), amrubicinol (AMR-OH), or the combination of CDDP and AMR-OH induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3/SN-38 cells.

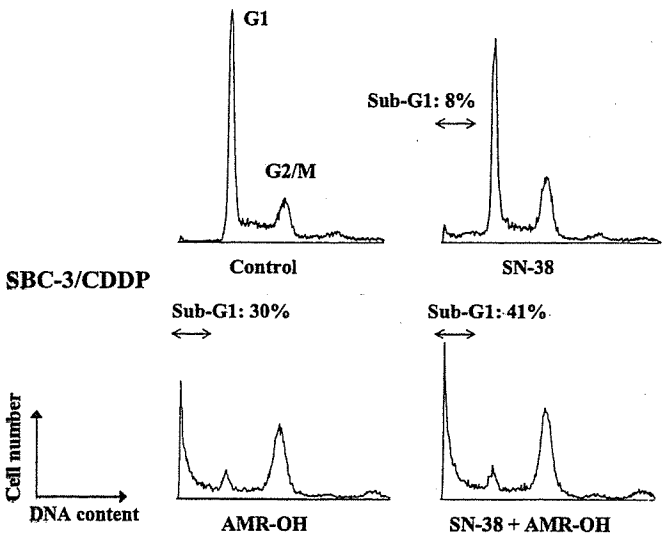

Figure 3. Effect of SN-38, amrubicinol (AMR-OH), or the combination of SN-38 and AMR-OH induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3/CDDP cells.

effect. At IC_{90}, the combination of amrubicinol and SN-38 showed an additive effect and that of amrubicinol and etoposide displayed a synergistic effect.

Combination effect of amrubicinol with SN-38 for SBC-3/CDDP and cisplatin for SBC-3/SN-38. CI values and the surviving fraction of SBC-3/CDDP cells treated by 96 h simultaneous exposure to amrubicinol and SN-38 are drawn in Fig. 1 (left). Based on IC_{50} values in resistant cells, the concentration ratio of amrubicinol and SN-38 was determined to be 5:1. CI values were 0.76±0.21 at IC_{70} and 1.0±0.35 at IC_{90}. Similarly, CI values and the surviving fraction of SBC-3/SN-38 cells treated by 96 h simultaneous exposure to amrubicinol and cisplatin are drawn in Fig. 1 (right). The concentration ratio of amrubicinol to cisplatin was 1:2.

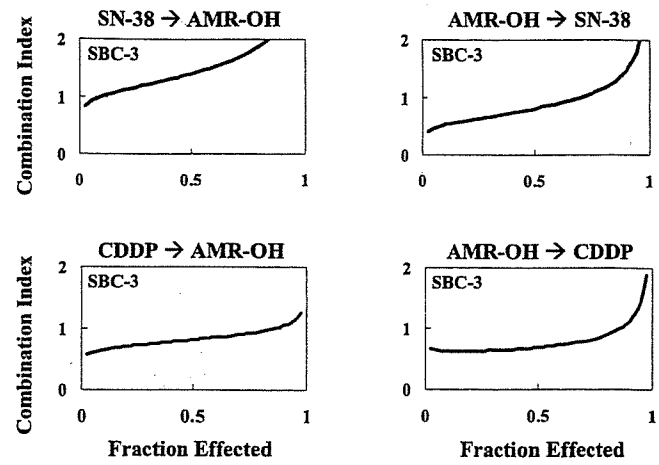

Figure 4. Combination index and surviving fraction of SBC-3 cells treated sequentially with SN-38 or cisplatin (CDDP) for 24 h followed by amrubicinol (AMR-OH) for 24 h and the reverse sequence.

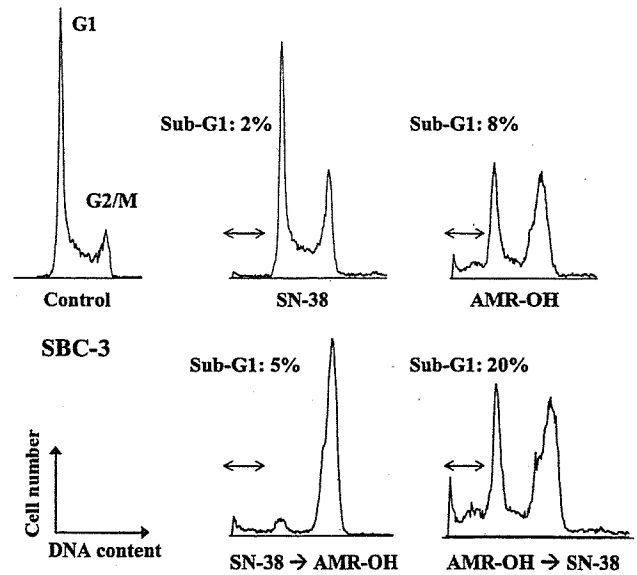

Figure 5. Effect of SN-38, amrubicinol (AMR-OH), SN-38 followed by AMR-OH, or AMR-OH followed by SN-38 induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3 cells.

CI values were 0.99±0.17 at IC_{70} and 0.89±0.24 at IC_{90}. Thus, the combination of amrubicinol with SN-38 showed synergistic or additive effects for cisplatin-resistant cells, and amrubicinol with cisplatin displayed a synergistic effect for SN-38-resistant cells. As shown in Fig. 2, an analysis of cell cycle traverse perturbations demonstrated that treating SBC-3/SN-38 cells with amrubicinol (50 nM) alone resulted in an accumulation of cells in the S+G2/M boundary and a measurable increase in the apoptotic cell population (sub-G1, 24%). Cisplatin (100 nM) alone increased apoptotic cells to 12%, however, the combination of these two drugs induced more apoptosis (35%). Similarly, treating SBC-3/CDDP cells with the combination of SN-38 (10 nM) and amrubicinol (50 nM) produced more apoptotic cells (sub-G1, 41%) than SN-38 alone (8%) or amrubicinol alone (30%) (Fig. 3).

Analysis of combination effect by exposure schedule of amrubicinol and SN-38 or cisplatin for SBC-3. CI values and

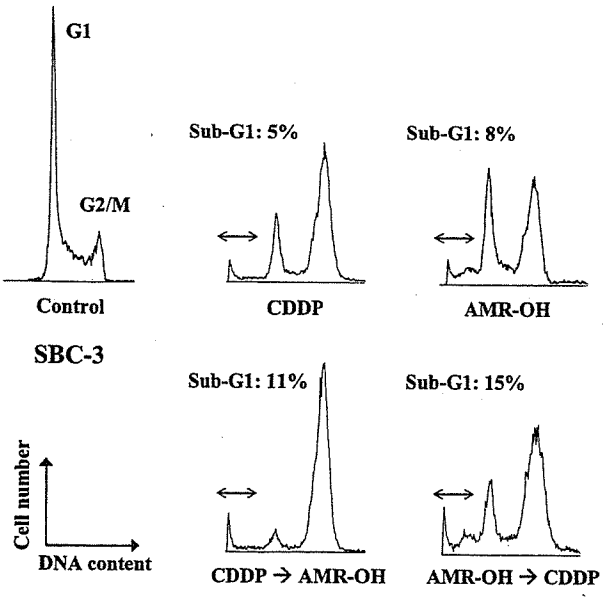

Figure 6. Effect of cisplatin (CDDP), amrubicinol (AMR-OH), CDDP followed by AMR-OH, or AMR-OH followed by CDDP induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3 cells.

the surviving fraction of SBC-3 cells treated sequentially with amrubicinol for 24 h followed by SN-38 or cisplatin for 24 h and those with a reverse sequence are shown in Fig. 4. The sequence of amrubicinol followed by SN-38 was more effective than the reverse sequence. As shown in Fig. 5, analysis of cell cycle traverse perturbations demonstrated that treatment of SBC-3 cells with amrubicinol alone resulted in an accumulation of cells in the S+G2/M boundary and a measurable increase in the apoptotic cell population (sub-G1, 8%). Treating the cells with SN-38 (5 nM) followed by amrubicinol (50 nM) resulted in no marked accumulation of cells at sub-G1 (5%), but the reverse sequence exposure produced a marked increase in apoptotic cells (20%). CI values after exposure to cisplatin followed by amrubicinol were 0.93±0.12 at IC_{70} and 1.0±0.06 at IC_{90}, and 0.86±0.15 at IC_{70} and 0.93±0.32 at IC_{90} for the reverse sequence. This combination of two drugs appears effective irrespective of sequence. Treatment with amrubicinol (50 nM) followed by cisplatin (500 nM) and the reverse sequence exposure increased the number of apoptotic cells (15% and 11%, respectively) as shown in Fig. 6.

Discussion

We have established adriamycin-resistant SBC-3/ADM (17), etoposide-resistant SBC-3/ETP (18), cisplatin-resistant SBC-3/CDDP (12), and SN-38-resistant SBC-3/SN-38 cells from SBC-3, which was derived from an untreated SCLC patient (11). Amrubicinol was found to be completely cross-resistant to adriamycin and etoposide in experiments using SBC-3/ADM and SBC-3/ETP cells (19). SBC-3/SN-38 cells had decreased topoisomerase I and II activity and over-expressed breast cancer-resistant protein compared to the SBC-3 cells (11). SBC-3/CDDP cells showed increased intracellular glutathione and glutathione S-transferase content and decreased intracellular accumulation of cisplatin (12). In the present study, SBC-3/SN-38 and SBC-3/CDDP retained sensitivity to amrubicinol. These results suggest that amrubicin may be effective for SCLC patients who were previously treated with cisplatin and irinotecan. In addition, the combination of amrubicinol and cisplatin showed a synergistic effect for SBC-3/SN-38 and that of amrubicinol and SN-38 displayed additive or synergistic effects for SBC-3/CDDP. In a phase II study, the combination of amrubicin and cisplatin was reported to be highly effective for untreated ED-SCLC (20). A combination of amrubicin and irinotecan was feasible and effective in some patients with relapsed non-small cell lung cancer in our phase I study (21). The present study suggests that combination of amrubicin and cisplatin or irinotecan is also worth evaluating in relapsed SCLC patients.

Amrubicin had additive effects in combination with cisplatin for several human tumor cells, including lung cancer cells, by isobologram analysis (22,23). The present study confirmed those results using SBC-3, as both simultaneous and sequential combinations of the two drugs displayed synergistic or additive effects by median-effect plot analysis. In addition, flow cytometric analysis showed that exposure of the two drugs produced an increase of apoptotic cells compared to that for each single agent. It was difficult to draw a conclusion about the effect of the combination of amrubicinol and SN-38. However, sequential exposure of amrubicinol followed by SN-38 may be considered for further studies since: i) CI values after simultaneous exposure of amrubicinol and SN-38 were 1.2 at IC_{70} (antagonistic) and 1.0 at IC_{90} (additive); ii) the effect of SN-38 followed by amrubicinol was antagonistic; and iii) CI values after sequential exposure of amrubicinol followed by SN-38 were 1.0 at IC_{70} (additive) and 1.1 at IC_{90} (antagonistic), and this sequence produced a marked increase in apoptotic cells. Amrubicinol had an additive effect with etoposide for T-cell leukemia cells and osteosarcoma cells, although the effects were antagonistic at IC_{70} and synergistic at IC_{90} for SBC-3 (22). To our knowledge, the combination of amrubicinol with paclitaxel, which had an antagonistic effect in this study, has not been reported. More cell lines should be investigated to further evaluate these combinations.

The mechanisms of drug interaction between amrubicinol and other drugs have not been elucidated. Flow cytometry data in the present study suggested the presence of apoptotic cells based on the sub-G1 peak. Biochemical analysis for apoptotic cell death should be carried out for further investigation. Yamauchi *et al* reported that cisplatin enhanced the topoisomerase II inhibitory effect of amrubicinol and amrubicinol enhanced the formation of cisplatin-induced DNA interstrand cross-links (23). A combination of topoisomerase I inhibitors and topoisomerase II inhibitors is thought reasonable because reciprocal enhancement of one enzyme in the resistant cell lines develops an inhibitory effect on the other enzyme (24). However, the effectiveness of a combination and administration schedule has been a controversial issue in clinical trials to date (25). Thus, additional research will be needed to establish a rationale for the combination of irinotecan and amrubicin.

The combination of irinotecan and cisplatin is accepted as the standard treatment for ED-SCLC (2). Concurrent

chemoradiotherapy consisting of cisplatin, etoposide and thoracic radiotherapy followed by cisplatin and irinotecan is considered to be very active in limited disease SCLC (26). The present study indicated that further studies are warranted on amrubicin alone and in combination with cisplatin or irinotecan in relapsed SCLC patients.

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ORIGINAL ARTICLE

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A phase I study of 3-day topotecan and cisplatin in elderly patients with small-cell lung cancer

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Abstract *Purpose*: The aim of this phase I study was to determine the maximum-tolerated dose (MTD) in elderly patients with small-cell lung cancer (SCLC). *Patients and methods*: Patients aged over 75 years with previously untreated SCLC were enrolled in this study. Both topotecan and cisplatin were administered on days 1–3 and repeated every 3 weeks. The starting dose of topotecan was 0.5 mg/m^2/day, while cisplatin was fixed at the dose of 20 mg/m^2/day. Patients with limited disease (LD) SCLC received thoracic irradiation after the completion of chemotherapy. *Results*: Twenty-one elderly patients were enrolled in this study and received a total of 59 cycles. The major hematological toxicity was neutropenia and non-hematological toxicities including diarrhea were generally mild and reversible. The MTD of topotecan was determined as 1.2 mg/m^2/day. The recommended phase II study dose of topotecan was determined as 1.0 mg/m^2/day with cisplatin 20 mg/m^2/day daily for 3 days. An objective response was observed in 6 of 10 patients (60%) with LD-SCLC and 6 of 11 (55%) with extensive disease (ED) SCLC. The median survival time in patients with LD-SCLC and those with ED-SCLC were 16.0 and 11.0 months, respectively. *Conclusion*: The combination chemotherapy of 3-day topotecan and cisplatin appears to be tolerable and effective in elderly patients with SCLC.

Keywords Small-cell lung cancer · Elderly patients · Topotecan · Cisplatin · Phase I study · 3-day schedule

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Introduction

The standard chemotherapy for extensive disease small-cell lung cancer (ED-SCLC) has been considered to be a combination of etoposide and cisplatin [1, 9, 11]. Recently, a randomized phase III study comparing a combination of irinotecan, one of the topoisomerase I inhibitors, and cisplatin with a standard combination of etoposide and cisplatin in patients with previously untreated ED-SCLC, demonstrated a significant survival benefit in a combination with irinotecan and cisplatin [18]. Thus, the combination of a topomerase-I inhibitor and cisplatin is an attractive strategy for the treatment of SCLC.

However, elderly patients were excluded from these previous trials [11, 18]. In general, elderly patients are

considered to have an increased risk of chemotherapy-related morbidity and mortality due to comorbid diseases, deterioration of organ functions, or poor performance status (PS) [6, 21]. In addition, frequent dose reductions due to excessive toxicities may be required in elderly patients because of poor functional reserves, resulting in an insufficient dose-intensity of the chemotherapy [27]. Regarding the toxicity profile of the irinotecan and cisplatin combination, one of the major toxicities seems to be high incidence of diarrhea (grade 2 or more: 44% [18]), which may lead to low treatment compliance in the elderly patients. Therefore, it is desirable to establish the optimal treatment for elderly patients with SCLC.

Topotecan is a semi-synthetic derivative of camptothecin, which is a potent inhibitor of the topoisomerase I enzyme and involved in DNA unwinding needed for DNA replication and transcription [8]. In the previous phase II monotherapy trial in the 5-day administration schedule, the overall response rate for previously untreated SCLCs was 39% [25]. Non-hematological toxicities were relatively mild. In particular, diarrhea has been reported to be rare, which is the dose-limiting toxicity (DLT) of irinotecan [14, 17]. Additionally, the safety and efficacy of a 3-day topotecan regimen have recently been reported in patients with ovarian cancer [4, 13], and this modified regimen seemed to be less toxic than a 5-day topotecan regimen [7] with a comparable antitumor activity in patients with ovarian cancer [4, 13]. These findings suggest that a 3-day topotecan might be safely administered to elderly patients with SCLC.

Based on these background data, we designed a phase I study of topotecan administered for three consecutive days in combination with cisplatin, a key drug for SCLCs in elderly patients with SCLC. The primary objective was to determine the maximum-tolerated dose (MTD) for each drug, with a secondary objective of assessing antitumor activity.

Patients and methods

Eligibility

The eligibility criteria for entry into this study were as follows: (1) pathologically proven SCLC, (2) age of 76 years or more, (3) no prior anticancer therapy,(4) PS of 0–2 on the Eastern Cooperative Oncology Group (ECOG) scale [20], (5) presence of evaluable lesions, (6) adequate reserves of hematological function (white blood cell [WBC] count $\geq$ 4,000/μl, neutrophil count $\geq$ 2,000/μl, hemoglobin level $\geq$ 9.5 g/dl, platelet count $\geq$ 10×10^4/μl), renal function (serum creatinine $\leq$ 1.5 mg/dl), hepatic function (total bilirubin $\leq$ 1.5 mg/dl, serum transaminases $<$ 2.5×upper limit of normal range) and pulmonary function ($PaO_2 \geq 60$ Torr at rest), and (7) acquisition of a written informed consent. Patients with symptomatic brain metastasis were excluded from the study. The baseline pretreatment evaluations included a complete history, physical examination, laboratory tests, a chest radiograph, computed tomography (CT) scans of the chest and abdomen, fibreoptic bronchoscopy, magnetic resonance imaging (MRI) of the brain, and a radionuclide bone scan, if medically indicated. The protocol was approved by the institutional review board of each participating institute.

Treatment scheme

Topotecan, diluted in 100 ml of physiological saline, was intravenously administered for 30 min on days 1–3. After the completion of the topotecan infusion, a fixed dose of cisplatin (20 mg/m^2/day), diluted in 300 ml of physiological saline, was intravenously administered over 1 h on the same days. The treatment was repeated every 3 weeks and six dose levels were planned (Table 1).

Four cycles of chemotherapy were planned. Patients were treated with at least two cycles of chemotherapy unless there was a disease progression, unacceptable toxicity in the first cycle, or withdrawal of their consent. Initiation of the next cycle of chemotherapy was delayed until recovery of the WBC count to 3,000/μl, the neutrophil count to $\geq$ 1,500/μl, the platelet count to $\geq 10\times10^4$/μl, hemoglobin $\geq$ 8.0 g/dl, and resolution of non-hematologic toxicities to $\leq$ grade 1. If grade 4 leukopenia, grade 4 neutropenia, or febrile neutropenia was noted, the use of granulocyte colony-stimulating factor (G-CSF) was permitted. Patients with limited-disease (LD)-SCLC received thoracic irradiation at a total of 45 Gy in 25 fractions after the completion of chemotherapy. In addition, patients achieving complete response received prophylactic cranial irradiation.

Assessment of toxicity and dose escalation

Toxicities were graded according to the National Cancer Institute-Common Toxicity Criteria Version 2.0. All treatment cycles were analyzed to determine DLT, although the decision to elevate the dose level was based on the toxicities in the first cycle. The DLT was defined as development of at least one of the following toxicities: any non-hematological toxicities $\geq$ grade 3 other than nausea, vomiting, and alopecia; grade 4 neutropenia or leukopenia lasting for 4 days or more; platelet count $\leq$ $1–10^4$/μl. At least three patients were scheduled to enter the study at each dose level and if all three patients developed the DLT, the dose level was determined to be

Table 1 Planned dose level

Dose levels	Cisplatin (mg/m^2/day)	Topotecan (mg/m^2/day)
1	20	0.5
2	20	0.65
3	20	0.8
4	20	1.0
5	20	1.2
6	20	1.4

the MTD. If one or two of the three patients experienced the DLT, three additional patients were subjected to the same dose level. The MTD was defined as a dose level that produced any of the DLTs developed in three or more patients among a maximum of six patients, and further dose escalation was not permitted. Dose escalation in the individual patient was not allowed. The recommended dose was defined as the dose level below the MTD for safe administration of the both drugs.

Assessment of efficacy

The response was evaluated according to the Standard Response Evaluation Criteria in Solid Tumors [28]. The time to progression and the overall survival time were calculated from the date of registration to this trial until the first document of disease progression and death, respectively, using the Kaplan–Meier method. Statistical analyses were performed using the STATVIEW 5.0 program (Brainpower, Calabasas, CA).

Results

Patient characteristics

Between November 2001 and September 2004, a total of 21 elderly SCLC patients were enrolled in this study (Table 2). In ED-SCLC patients, most frequent metastatic sites were the liver and adrenal gland. A total of 59 cycles were administered, with median number of three cycles per patients (range 1–4). Seven of the 10 LD-SCLC patients received thoracic irradiation after completion of chemotherapy with a median-delivered dose of 45 Gy. One patient received only one cycle of chemotherapy because of withdrawal of consent. All patients and cycles were assessable for toxicity and response.

Hematological toxicity

The hematological toxicities in 21 patients are listed in Table 3. The main toxicity was neutropenia, which was observed in 54 (91.5%) of 59 cycles. G-CSF was required in 34 (58%) cycles for grade 4 neutropenia (31 cycles) or febrile neutropenia (three cycles). Grade 4 anemia was observed in seven (12%) cycles, and blood transfusion was required in four cycles at dose levels 3 and 5. Grade 2 or 3 thrombocytopenia was frequently observed and platelet transfusion was required in one cycle at dose level 5, however, no severe hemorrhage complications were experienced.

Table 2 Patient characteristics

No. of patients	21
Age	
Median (range)	78 (76–82)
Gender	
Male	19
Female	2
Performance status	
0	5
1	13
2	3
Stage	
Limited disease	10
Extensive disease	11

Non-hematological toxicity

Table 4 shows non-hematological toxicities of grade 2 or greater in all treatment cycles. Diarrhea was extremely mild and grade 1 diarrhea occurred in 7 (12%) of 59 cycles and no grade 2 or more diarrhea was observed in this study. Febrile neutropenia was experienced in one and two cycles at dose levels 3 and 5, respectively, however, it was reversible with appropriate supportive care including G-CSF and antibiotics. Grade 3 hepatic dysfunction and grade 4 hyponatremia occurred in one cycle each, and these toxicities were considered to be the DLT. However, these conditions spontaneously recovered. There were no treatment-related deaths.

Maximum-tolerated dose

Dose limiting toxicity was observed in one of six patients at dose level 3 (hepatic toxicity), and in three of six patients at dose level 5 (febrile neutropenia, persistent neutropenia, and hyponatremia). Thus, we determined the MTD of 3-day topotecan and cisplatin to be 1.2 and 20 mg/m^2/day, respectively (dose level 5). The recommended doses were considered to be 1.0 mg/m^2/day for topotecan and 20 mg/m^2/day for cisplatin (dose level 4).

Antitumor activity

An objective response was observed in 6 (60%) of 10 patients with LD-SCLC and 6 (55%) of 11 patients with ED-SCLC. The median follow-up time of the surviving patients was 11.0 months, and the median survival time was 12.8 months. When stratified by disease extent, the median survival times in patients with LD-SCLC and those with ED-SCLC were 16.0 and 11.0 months, respectively.

Discussion

The present phase I study demonstrated that the combination chemotherapy of 3-day topotecan and cisplatin was well tolerated in elderly SCLC patients. The major toxicity in our study was myelosuppression, whereas diarrhea was rarely observed. All the toxicities were reversible and no life-threatening toxicities occurred.

Table 3 Hematological toxicity of grade 2 or greater (all cycles)

		Dose levels				
		1	2	3	4	5
No. of treated patients		3	3	6	3	6
No. of cycles evaluated		9	5	19	7	19
	Grades	No. of cycles (%)				
Leukopenia	2	4 (44)	2 (40)	7 (37)	4 (57)	10 (53)
	3	1 (11)	1 (20)	10 (53)	0	6 (32)
	4	0	0	0	0	2 (11)
Neutropenia	2	0	1 (20)	2 (11)	1 (14)	0
	3	4 (44)	3 (60)	6 (32)	1 (14)	9 (47)
	4	3 (33)	1 (20)	10 (53)	3 (43)	10 (53)
Anemia	2	3 (33)	0	6 (32)	2 (29)	3 (16)
	3	1 (11)	2 (40)	3 (16)	2 (29)	3 (16)
	4	0	0	2 (11)	0	5 (26)
Thrombocytopenia	2	3 (33)	2 (40)	4 (21)	0	2 (11)
	3	2 (22)	2 (40)	6 (32)	0	11 (58)
	4	0	0	0	0	0

Table 4 Non-hematological toxicity of grade 2 or greater (all cycles)

		Dose levels				
		1	2	3	4	5
No. of treated patients		3	3	6	3	6
No. of cycles evaluated		9	5	19	7	19
	Grades	No. of cycles (%)				
Nausea/vomiting	2	2 (22)	1 (20)	2 (11)	2 (29)	2 (11)
	3	2 (22)	0	6 (32)	0	2 (11)
Fatigue	2	1 (11)	1 (20)	0	0	0
	3	0	0	0	0	8 (42)
Hepatotoxicity	2	0	0	0	1 (14)	0
	3	0	0	1 (5)	0	0
Infection	3	1 (11)	0	2 (11)	1 (14)	2 (11)
Febrile Neutropenia	3	0	0	1 (5)	0	2 (11)
Hyponatremia	4	0	0	0	0	1 (5)

The MTDs for topotecan and cisplatin were determined to be 1.2 and 20 mg/m^2/day, respectively (dose level 5), and this regimen yielded a favorable antitumor activity.

It is of note that diarrhea was extremely mild in our regimen without any grade 2 or over. Diarrhea was a major toxicity in the irinotecan and cisplatin arm of the

Table 5 Response

	Dose level					Total
	1	2	3	4	5	
LD-SCLC						
No. of patients evaluated	1	2	3	1	3	10
CR	1	0	2	1	0	4 (40%)
PR	0	0	0	0	2	2 (20%)
NC	0	2	1	0	0	3 (30%)
PD	0	0	0	0	1	1 (10%)
ED-SCLC						
No. of patients evaluated	2	1	3	2	3	11
CR	0	0	0	0	0	0 (0%)
PR	1	0	2	1	2	6 (55%)
NC	1	1	0	1	1	4 (36%)
PD	0	0	1	0	0	1 (9%)

LD-SCLC limited disease small-cell lung cancer, *ED-SCLC* extensive disease small-cell lung cancer, *CR* complete response, *PR* partial response, *NC* no change, *PD* progressive disease

recent randomized phase III study. Indeed, grade 2 or more diarrhea occurred in 44% of the evaluable patients [18]. Topotecan has the advantage of a lower incidence of diarrhea compared to irinotecan when combined with cisplatin. However, Lilenbaum et al.[12] also demonstrated in a phase I study of topotecan combined with cisplatin that grade 2 diarrhea occurred in 3 (9.7%) of 31 patients despite the fact that no grade 3 or 4 diarrhea was experienced. In addition, Ardizzoni et al.[3] reported grade 3 or 4 diarrhea to be 4% in a phase II trial of topotecan with cisplatin. Accordingly, the 3-day administration schedule in the present study may be superior to prevent diarrhea.

In the previous phase I studies of topotecan and cisplatin, the major toxicity was myelosuppression [12, 16, 22–24]. In a phase I study of 5-day topotecan with cisplatin conducted by Miller et al.[16], dose-limiting grade 4 neutropenia lasting for more than 7 days occurred in three (30%) of nine patients, whereas our 3-day-schedule regimen did not show such a durable toxicity. Additionally, in a phase II study comparing a 3-day regimen of topotecan and cisplatin with a 5-day regimen, the incidence of grade 3 or more leukopenia was somewhat lower in the former regimen (22 and 33%, respectively) [26]. These observations suggest that a 3-day topotecan regimen may be less toxic than a 5-day one, although other clinical factors possibly affected the difference of the toxicity profiles. Furthermore, the frequency of neutropenia in our trial was almost comparable with that in the irinotecan and cisplatin arm of the randomized trial [18], and that in the combination chemotherapy of carboplatin and etoposide in elderly patients with SCLC [19]. Thus, our regimen is considered to be safely administrable in terms of both hematological and non-hematological toxicity when compared with the previous results.

With regard to the efficacy, our regimen seems to have potential antitumor activity in elderly patients with SCLC, with response rates of 60% in LD-SCLC and 55% in ED-SCLC. In addition, the median survival times for LD- and ED-SCLC were 16.0 and 11.0 months, respectively. In the previous clinical trials, median survival times in the treatment of elderly LD- and ED-SCLCs were reported to be 12–15 and 9–11 months, respectively, with combination chemotherapy consisting of carboplatin and etoposide [5, 10, 15, 19]. Ardizzoni et al.[2] recently conducted a phase II study of cisplatin and etoposide in elderly patients with LD- and ED-SCLC. They demonstrated that the overall response rate and survival time were 60.0% and 9.5 months, respectively. The clinical outcome in our study seems to be comparable with these studies, suggesting that this regimen has considerable antitumor activity in elderly patients with SCLC. Because of the small sample size in this study, it is necessary to verify the efficacy of this regimen in a subsequent phase II study.

In conclusion, combination chemotherapy consisting of topotecan and cisplatin on days 1–3 is well tolerated for elderly patients with SCLC, which seems to show reasonable efficacy. The phase II study of this regimen is now under investigation.

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