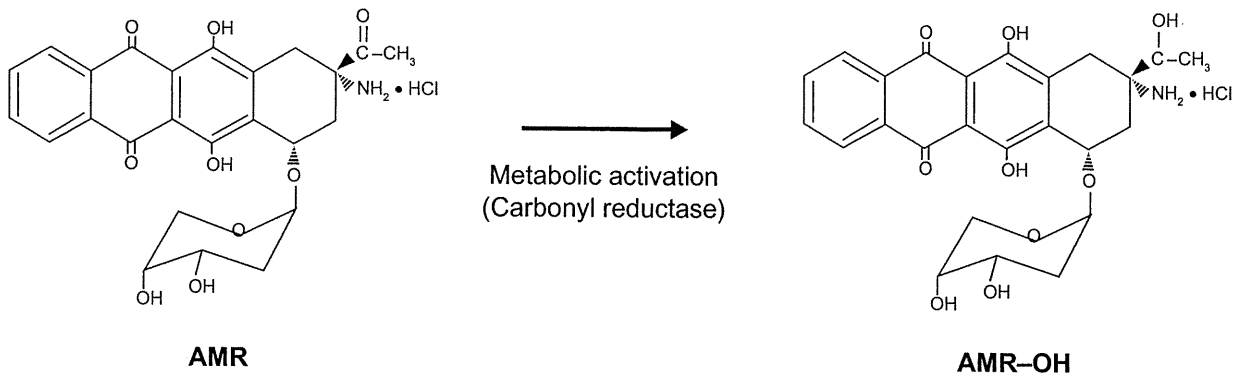

AMR **AMR–OH**

Figure 1. Chemical structures of amrubicin and amrubicinol.

tissues, including the heart. In addition, by measuring the concentrations of AMR-OH in seven human tumor xenografts after the administration of AMR, a good correlation was found between the level of AMR-OH in the tumor and the efficacy of AMR *in vivo*.[38] Thus, AMR appears to be a very promising antitumor agent: its potent antitumor activity is due to high levels of the active metabolite in the tumor, and its non-hematological toxicities, mainly cardiac toxicities, can be easily controlled because of the restricted distribution of the active metabolite in non-tumor tissues.

In vitro and *In vivo* Studies

Several studies have reported a comprehensive assessment of the clinical uses of AMR in combination with chemotherapeutic agents analyzed by the isobologram method[39] or by the combination index values.[40] We reported *in vitro* studies in the SCLC cell line SBC-3 and in the NSCLC cell line Ma-1—that CDDP enhanced the effect of AMR-OH, and that AMR-OH enhanced the formation of CDDP-induced DNA interstrand cross-links.[41] Another group reported the combination effects of AMR with other anticancer agents analyzed by the isobologram method in the T-cell leukemia cell line MOLT-3 and the human osteosarcoma cell line MG-63.[42] In MOLT-3 cells, AMR-OH had additive effects with bleomycin, VP-16, doxorubicin, CDDP, mitomycin-C, 4-hydroperoxy ifosfamide, 5-fluorouracil, cytarabine, and vincristine, whereas it had mainly protective (marked antagonistic) effects with methotrexate. In MG-63 cells, AMR-OH had additive effects with bleomycin, VP-16, doxorubicin, CDDP, mitomycin-C, 4-hydroperoxy ifosfamide; mainly sub-additive (mild antagonistic) effects with 5-fluorouracil and cytarabine; and mainly protective (marked antagonistic) effects with vincristine and methotrexate. Takigawa et al reported that AMR-OH was completely cross-resistant to doxorubicin and VP-16 in experiments using the doxorubicin-resistant SCLC cell line SBC-3/ADM and the VP-16-resistant SCLC cell line SBC-3/ETP.[43] Simultaneous exposure of the irinotecan (CPT-11)-resistant SCLC cell line SBC-3/SN-38 to AMR-OH and CDDP showed a synergistic effect when analyzed by the combination index values. Simultaneous exposure of the CDDP-resistant SCLC cell line SBC-3/CDDP to AMR-OH resulted in synergistic effects.[44] In addition, multi-drug combination effects have been reported for AMR-OH in combination with chemotherapeutic agents *in vitro* models when analyzed by the combination index values and in human lung cancer xenograft models.[45] In these experiments, human SCLC cell lines, NSCLC cell lines, a breast cancer cell line, and human gastric cancer cell lines were simultaneously exposed to two agents for 3 days. AMR-OH showed synergistic effects for the simultaneous use of CPT-11, CDDP, gefitinib and trastuzumab; additive effects with vinorelbine; and antagonistic interactions with gemcitabine. As for AMR, synergistic effects were found for simultaneous use with CPT-11, gefitinib and trastuzumab; and additive effects were demonstrated with CDDP and vinorelbine. In human lung cancer xenograft models, AMR administered intravenously at 25 mg/kg substantially prevented the growth of five out of six human lung cancer xenografts established in athymic nude mice. Synergistic effects were obtained for the simultaneous

use of AMR-OH with CDDP, CPT-11, gefitinib and trastuzumab. The combination of AMR-OH with gemcitabine was antagonistic. As just described, the combination with AMR and some chemotherapeutic agents has theoretical advantages and have proven anticancer efficacy. A clinical outcome includes both antitumor response and normal tissue toxicity from a variable drug exposure, whereas *in vitro* models represent only antitumor response. Further studies are warranted on AMR in combination with chemotherapeutic agents in clinical settings.

Mechanisms of Action of Anthracyclines and AMR

DNA topoisomerases I and II are functionally related nuclear enzymes that, in concert, catalyze the relaxation of supercoiled chromosomal DNA during DNA replication. The relaxation of DNA by topoisomerase I or II involves the transient single or double strand breakage of DNA, followed by strand passage and relegation of the DNA strand. They are extensively involved in DNA replication, transcription, and recombination, and in sister chromatin segregation, and as such are essential in maintaining cell viability.[46] Mammalian DNA topoisomerase II is the primary target of a number of antitumor agents such as doxorubicin, daunorubicin, VP-16 and amsacrine.[47] These agents interfere with the breakage–reunion reaction of DNA topoisomerase II by trapping a covalent enzyme–DNA complex, termed "the cleavable complex", in which DNA strands are broken and their 5′ termini are covalently linked to the protein. AMR and AMR-OH also stabilize the topoisomerase II-DNA complex,[36] but the mechanisms of cell killing by AMR and AMR-OH are not understood.

Combination Therapy with Topoisomerase I and II Inhibitors

Studies have shown that the use of a combination of topoisomerase I and II inhibitors completely arrests both DNA and RNA synthesis, which results in synergistic cytotoxicity. Preclinical studies have demonstrated that resistance to CPT-11, a topoisomerase I inhibitor, is often accompanied by the upregulation of topoisomerase II, causing hypersensitivity to agents that target topoisomerase II.[48–50] Consequently, the scheduling of therapy with a combination of CPT-11 and a topoisomerase II inhibitor is critical for success:[51] sequential administration of CPT-11 followed by a topoisomerase II inhibitor led to synergistic cytotoxicity, while concurrent administration led to antagonism.[52]

Clinical information on the combination of topoisomerase I and II inhibitors in the treatment of patients with SCLC is limited. Masuda et al conducted a phase II study on refractory or relapsed SCLC.[21] Twenty-five patients were treated at 4-weekly intervals with CPT-11 at a dose of 70 mg/m^2 on days 1, 8, and 15, plus VP-16 at a dose of 80 mg/m^2 on days 1–3, with granulocyte colony-stimulating factor (G-CSF) support. The overall RR was 71% and the MST was 8.9 months. Another phase II study was reported by Goto et al.[53] Forty patients with sensitive relapsed SCLC were treated with CPT-11 at a dose of 90 mg/m^2 on day 1 in weeks 2, 4, 6, and 8, CDDP at a dose of 25 mg/m^2 on day 1 weekly for 9 weeks, and VP-16 at a dose of 60 mg/m^2 on days 1–3 of weeks 1, 3, 5, 7 and 9, with G-CSF support. The overall RR was 78% and the MST was 11.8 months. Quoix et al reported a phase II study investigating the efficacy and safety of topotecan in combination with either CDDP or VP-16 in untreated extensive disease (ED)-SCLC.[54] Patients were randomized to treatment with T/C (topotecan at 1.25 mg/m^2 on days 1–5, CDDP at 50 mg/m^2 on day 5) or T/E (topotecan at 0.75 mg/m^2 on days 1–5, VP-16 at 60 mg/m^2 on days 1–5) every 21 days. The RRs were similar for the T/C (63.4%) and the T/E (61.0%) groups. The MST was 9.6 months for the T/C group and 10.1 months for the T/E group. Furthermore, Mok et al conducted a phase I–II study of the sequential administration of topotecan and oral VP-16, with alternation of the drug sequence with each consecutive cycle, and compared the hematologic toxicity between the two sequences.[55] Thirty-six patients (21 with limited disease and 15 with extensive disease) received a total of 173 courses of sequential combination chemotherapy (topotecan followed by VP-16, and VP-16 followed by topotecan). There was no significant difference in hematologic toxicity between the two sequences. The combination of topoisomerase I and II inhibitors was considered highly effective and well tolerated in the treatment of SCLC.

Pharmacokinetics and Pharmacodynamics

In a pharmacokinetic study examining the time-concentration profiles of AMR and AMR-OH, the plasma concentration curves fitted a three-compartment open model.[37] AMR was metabolized to AMR-OH by human tumor cells, and substantial amounts of AMR-OH were found in cells after a five-hour incubation with AMR in several cancer cell lines.[31] AMR-OH is less susceptible than AMR to further metabolism or is retained in tissues for a longer period.[37] It was also found that the ratio of AMR-OH to AMR plasma levels was approximately 0.1, from 1 h after administration.[37] Although the plasma concentration curve of AMR exhibited a high peak in the α/β phase and a downward slope in the γ phase, that of AMR-OH exhibited a slight or low peak in the α/β phase and a continuous long plateau in the γ phase. The half-lives in the terminal phase ($T1/2\gamma$) of AMR and AMR-OH, after administration of 40 mg/m^2 AMR on day 1, were 6.2 ± 2.0 and 16.2 ± 4.66 h, respectively.[56] Another study reported the $T1/2\gamma$ of AMR and AMR-OH, after administration of 30 mg/m^2 AMR on day 3, to be 2.2 ± 0.19 and 23.2 ± 18.26 h, respectively.[57]

The pharmacodynamic profiles in a phase I trial showed the relationships between the area under the concentration–time curve (AUC), the maximum drug concentration (C_{max}) of plasma AMR, and clinical efficacy. The AUC_{0-24} of AMR was significantly correlated with the AUC of AMR-OH.[56] The AUC and C_{max} of plasma AMR were related to the duration of grade 4 neutropenia.[58] Another pharmacological study reported a significant relationship between the grade of leukopenia and the AUC of AMR-OH.[59] Previously, we reported a significant relationship between hematological toxicity and the plasma trough concentration of AMR-OH.[60] Significant relationships were observed between the levels of AMR-OH on day 4 and the toxicity grades of leukopenia, neutropenia, and anemia ($P = 0.018$, $P = 0.012$, and $P = 0.025$, respectively). The thrombocytopenia grade exhibited a tendency towards correlation with AMR-OH levels on day 4 ($P = 0.081$). The plasma concentration of AMR-OH on day 4 was positively correlated with percent change in neutrophil count in the group comprising all patients, as well as in patients treated with AMR alone and in patients co-administered CDDP. The plasma concentration of AMR or AMR-OH correlated with hematological toxicity in patients treated with AMR. Such pharmacological studies might facilitate the prediction of hematological toxicity.

Clinical Trials with Amrubicin Hydrochloride Monotherapy

Phase I studies

At first, a dose escalation study of AMR given on day 1 of every 3-week period was performed in a phase I setting for 19 patients with advanced cancer.[61] Twenty-nine evaluable courses of treatment were conducted in groups, with doses increasing from 10 to 130 mg/m^2. Myelosuppression was the dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD) was 130 mg/m^2. The recommended dose (RD) and schedule for a phase II trial was 100 mg/m^2 every 3 weeks.

Next, as a 5-min intravenous infusion for three consecutive days, a phase I–II study was conducted on patients with previously untreated NSCLC.[62] The MTD was 50 mg/m^2/day and the DLTs were leukopenia, neutropenia, thrombocytopenia, and gastrointestinal complications. The RD in the phase II study was 45 mg/m^2 for three consecutive days every 3 weeks. A phase I study for refractory or relapsed lung cancer (NSCLC or SCLC) patients was conducted by Okamoto et al.[56] Fifteen patients were treated with AMR at doses of 30, 35, or 40 mg/m^2 on three consecutive days every 3 weeks. Grade 4 neutropenia was observed in 67% of patients, and the MTD and RD were determined as 40 mg/m^2 and 35 mg/m^2, respectively. Similarly, Igawa et al conducted a dose-escalation study of second-line and third-line settings for SCLC.[63] The RDs were determined to be 40 mg/m^2 and 35 mg/m^2, respectively.

Phase II studies

Yana et al conducted a phase II study on previously untreated ED-SCLC patients.[64] AMR was administered intravenously at a dose of 45 mg/m^2/day on three consecutive days every 3 weeks. Of the 33 patients, the overall RR and MST were 75.8% and 11.7 months, respectively. The 1-year and 2-year survival rates were 48.5% and 20.2%, respectively; however, hematologic toxicities were severe: grade

3/4 neutropenia, anemia, and thrombocytopenia were observed in 84.8%, 78.8%, and 39.4% of patients, respectively.

The efficacy and safety of AMR in patients with previously treated SCLC have been demonstrated in several phase II studies (Table 1). In Japan, five phase II studies have been conducted at different doses of AMR for relapsed SCLC. In the first three studies described below, AMR was administered as a single agent at 40 mg/m^2 for three consecutive days.[65–67] In the first study, conducted by our group, 19 patients were treated with AMR. The RRs in 7 sensitive and 12 refractory relapse patients were 43%, and 33%, respectively.[65] In the second study, conducted by Onoda et al, the RR and MST in sensitive relapse and refractory relapse patients were 52% and 11.6 months, and 50% and 10.3 months, respectively.[66] In the third study, a randomized phase II trial comparing topotecan and AMR was conducted.[67] Sixty patients were randomly assigned to either AMR or topotecan, and 59 (36 sensitive relapse and 23 refractory relapse patients) were evaluable. For AMR treatment, the RRs of overall, sensitive relapse, and refractory relapse patients were 38%, 53%, and 17%, respectively. The median progression-free survival time (PFS) and MST were 3.5 months and 8.1 months, respectively. In the fourth study, conducted by Kato et al, 45 mg/m^2 of AMR was administered on days 1–3, every 3 weeks.[68] Thirty-four patients were treated with AMR, and there were four complete responses (CRs) and 14 partial responses (PRs), with an RR of 53%. The RR and MST among sensitive relapse and refractory relapse patients were 50% and 10.4 months, and 60% and 6.8 months, respectively. The fifth study was conducted by Kaira et al, in which 35 mg/m^2 of AMR was administered to both SCLC and NSCLC patients.[28] In this study, 29 relapsed SCLC patients were enrolled, and the RR and MST among sensitive relapse and refractory relapse patients were 60% and 12.0 months, and 37% and 11.0 months, respectively. These five studies resulted in an RR of the sensitive relapse patients in this fifth report of 50.0%–53.0%, and that of refractory relapse patients of 17.0%–60.0%. AMR is a promising therapeutic for chemotherapy-sensitive relapse patients as well as for chemotherapy-refractory relapse patients. To support the efficacy for chemotherapy-refractory relapse patients, a phase II study of AMR in patients with SCLC that is refractory or relapsed within 90 days of completing previous treatment is ongoing in Japan.

Table 1. Phase II studies of amrubicin monotherapy for recurrent SCLC.

Authors	Dose (mg/m^2)	n	RR (%)	PFS (months)	MST (months)
Sensitive relapse					
Kudoh et al[65]	40	7	42.8	NA	NA
Onoda et al[66]	40	44	52	4.2	11.6
Inoue et al[67]	40	17	53	3.9	9.9
Kato et al[68]	45	24	50	NA	10.4
Kaira et al[28]	35	10	60	4	12
Jotte et al[69]	40	50	44	4.6	9.3
Refractory relapse					
Kudoh et al[65]	40	12	33.3	4	8.3
Onoda et al[66]	40	16	50	2.6	10.3
Inoue et al[67]	40	12	17	2.6	5.3
Kato et al[68]	45	10	60	NA	6.8
Kaira et al[28]	35	19	36.8	4	11
Ettinger et al[29]	40	75	21	3.2	6

Abbreviations: RR, response rate; PFS, progression free survival; MST, median survival time.

Two phase II studies have been conducted outside Japan. In the first study, conducted in the USA, 76 sensitive relapse patients were randomly assigned to either AMR or topotecan.[69] The RR, the median PFS and MST for AMR were 36%, 4.3 months and 9.3 months, respectively. In the second study, 75 refractory relapse patients were treated with 40 mg/m^2 AMR on three consecutive days every 3 weeks.[29] The RR and MST were 21% and 6.0 months, respectively. The RRs and MSTs in these two studies conducted outside Japan were considerably lower than those of the Japanese phase II studies.

Interestingly, there were two phase II studies comparing topotecan and AMR conducted in Japan and USA.[67,69] In the Japanese study, topotecan was administered at a dose of 1.0 mg/m^2 on days 1–5, every 3 weeks. For topotecan treatment, the RRs of overall, sensitive relapse and refractory relapse patients were 13%, 21%, and 0%, respectively. The median PFS and MST were 2.2 months and 8.4 months, respectively. AMR had significantly better overall RR rates than topotecan ($P = 0.039$). However, the hematologic and nonhematologic toxicities worse than grade 3 were more frequent in the AMR arm. In terms

of overall survival, there was no statistical difference between topotecan and AMR. However, a significant difference in overall survival was observed between patients treated with AMR and those without AMR ($P < 0.001$). The USA study was conducted only for sensitive relapse patients, and topotecan was administered at a dose of 1.5 mg/m^2 on days 1–5, every 3 weeks. The RR, the median PFS and MST for topotecan were 8%, 3.5 months, and 8.9 months, respectively. AMR gave significantly better overall RR rates than topotecan ($P < 0.012$). The most common grade ≥3 adverse events with AMR vs. topotecan were neutropenia (53% vs. 74%), thrombocytopenia (31% vs. 52%) and leukopenia (27% vs. 30%). Statistical analyses in terms of overall survival between topotecan and AMR were not reported. As a result, AMR had better overall RR rates than topotecan. There is no difference between topotecan and AMR in the terms of overall survival. However, considering subsequent chemotherapy after the enrollment in these studies, AMR may have more influence than topotecan on overall survival.

Side Effects

The most frequent toxicity was myelosuppression. Previous phase II studies of AMR monotherapy for treated SCLC found that treatment was associated with a high incidence of bone marrow suppression or grade 3 or 4 hematologic toxicity.[29,66,67] These toxicities comprised neutropenia (83%), thrombocytopenia (20%), and anemia (33%) in Onoda et al's report; neutropenia (93%), thrombocytopenia (28%), and anemia (21%) in Inoue et al's report; and neutropenia (66.7%), thrombocytopenia (40.6%), and anemia (30.4%) in Ettinger et al's report. Consistent with these results, the major adverse events in our own study were grade 3 or 4 hematologic toxicities including neutropenia (85%), leukopenia (85%), thrombocytopenia (32%), and anemia (42%).[65]

Non-hematologic toxicities were generally mild, except for grade 3 febrile neutropenia. Onoda et al described the most frequent grade 3 or 4 non-hematologic toxicities as anorexia (15%), asthenia (15%), hyponatremia (8%), nausea (5%), and febrile neutropenia (5%).[66] In Inoue et al's report, the most frequent grade 3 or 4 non-hematologic toxicities were fatigue (17%), febrile neutropenia (14%), infection (10%), anorexia (7%), stomatitis (3%), and nausea (3%).[67] According to Ettinger et al, the most common grade 3 or 4 non-hematologic toxicity was fatigue (21.7%).[29] Grade 3 or 4 febrile neutropenia was seen in 11.6%. No cardiotoxicity, except for one transient atrial fibrillation, was observed among these three reports. No treatment deaths occurred in our study,[65] or in that of Onoda et al.[66] However, there was one treatment-related death, resulting from neutropenic infection, in the AMR arm of Inoue et al's study,[70] and there was one patient death each of pulmonary hemorrhage, acute myocardial infarction, and interstitial lung disease in the Ettinger et al study.[29]

Clinical Trials with Amrubicin-based Combination Therapy

Rationale for combination therapy

As shown in Table 2, AMR has been used in clinical trials in double combination regimens. There is a clear need for non-cross-resistant therapeutic options. *In vitro* antitumor synergy with many chemotherapeutic agents may indicate AMR as an ideal candidate for use in combination therapy.

Topoisomerase I Inhibitors and Amrubicin

CPT-11 and AMR have been used in three phase I studies of patients with advanced NSCLC.[57,71,72] In the first study,[57] both drugs were administered on days 1 and 8, and the MTDs of CPT-11 and AMR were 100 and 45 mg/m^2, respectively. This level had 3 of 4 patients with DLTs (persistence of grade 4 neutropenia and grade 4 leukopenia, persistence of grade 4 neutropenia, and grade 3 febrile netropenia). The RDs of CPT-11 and AMR were 100 and 40 mg/m^2, respectively. In the second study,[71] patients were treated at 3-weekly intervals with dose-escalated AMR (days 1–3) plus a fixed dose of 60 mg/m^2 CPT-11 (days 1 and 8). The 30 mg/m^2 AMR dose was one dose level above the MTD, since diarrhea and leukopenia were the DLTs. The RDs are 60 mg/m^2 of CPT-11 (days 1 and 8) and 25 mg/m^2 of AMR (days 1–3), administered every 3 weeks. The third study was a dose escalation study of AMR in combination with fixed-dose CPT-11 in patients with

Table 2. Clinical trials with amrubicin-based combination therapy.

Authors	Histology	Phase	Patient selecion	Drugs	Schedule (day)	Interval (weeks)	MTD (mg/m²)	RD (mg/m²)
Topoisomerase I inhibitors								
Hotta et al[71]	NSCLC	I	untreated or treated	AMR	1, 8	3	45	40
				CPT-11	1, 8		100	100
Yanaihara et al[57]	NSCLC	I	untreated	AMR	1–3	3	30>	25
				CPT-11	1, 8		60	60
Oshita et al[72]	NSCLC	I	untreated	AMR	1–3	2	40	35
				CPT-11	1		60	60
Shibayama et al[58]	SCLC	I	untreated or treated	AMR	3–5	4	40	35
				Topotean	1–5		0.75	0.75
Nogami et al[73]	SCLC	II	untreated or treated	AMR	3–5	3	–	35
				Topotean	1–5		–	0.75
Platinum agents								
Yoshimura et al[74]	NSCLC	I	untreated	AMR	1–3	3	30	30
				CDDP	1		80	80
Ikeda et al[75]	NSCLC	I	treated	AMR	1–3	3–4	30	25
				CDDP	1–3		20	20
Ohe et al[76]	SCLC	I/II	untreated	AMR	1–3	3	45	40
				CDDP	1		60	60
Fukuda et al[77]	SCLC	I	untreated	AMR	1–3	3	40	35
				CBDCA	1		AUC5	AUC5
Inoue et al[70]	Elderly SCLC	I	untreated	AMR	1–3	3	40	35
				CBDCA	1		AUC4	AUC4

Abbreviations: MTD, maximum tolerated dose; RD, recommended dose; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; AMR, amrubicin; CPT-11, irinotecan; CDDP, cisplatin; CBDCA, carboplatin; AUC, area under curve.

ED-SCLC reported by Oshita et al.[72] Previously untreated patients with ED-SCLC were treated with CPT-11 at 60 mg/m² on day 1 and dose-escalated AMR on days 1–3, with prophylactic subcutaneous G-CSF on days 5–9 every 2–3 weeks. At 40 mg/m² AMR, DLTs such as grade 4 neutropenic fever were observed, and therefore this dose level was defined as the MTD, with an overall RR of 100%.

A phase I study of combination topotecan and AMR therapy in SCLC patients with relapsed or ED-SCLC was reported by Shibayama et al.[58] Topotecan and AMR were administered on days 1–5 and on days 3–5 every 4 weeks, respectively. DLTs (grade 4 neutropenia lasting for more than 4 days, grade 3 febrile neutropenia, or grade 4 thrombocytopenia) were observed at 0.75 mg/m² topotecan and 40 mg/m² AMR, and thus these were determined to be the MTDs. An objective response was observed in six patients (67%). The phase II study of the same regimen every 3 weeks for chemo-naïve or relapsed SCLC was reported.[73] The RRs were obtained in 23 (74%) of the 31 chemo-naïve and 12 (43%) of the 28 relapsed patients. Myelosuppresion was the principal toxixity with grade 4 leukopenia, neutropenia, thrombocytopenia and anemia of 46%, 80%, 25% and 7%, respectively. Grade 3–4 febrile neutropenia was observed in 41% of the patients, of whom one patient further developed Grade 5 septic shock. Other grade 3 or greater non-hematological toxicities included diarrhea, pneumonitis, vomiting, fatigue and hyponatremia in 2%, 3%, 5%, 9% and 2%, respectively. One patient each developed fatal diarrhea and pneumonitis. At the time of data analysis with a median follow-up time of 43.2 months, MST and median PFS were 14.9 and 5.3 months in the chemo-naïve patients and 10.2 and 5.1 months in the relapsed patients, respectively. Other ongoing studies include a phase II study of CPT-11 plus CDDP followed by AMR in patients with ED-SCLC, and a phase I–II study of AMR and CPT-11 in patients with advanced SCLC. The combination of topoisomerase I Inhibitors and AMR seemed effective for SCLC, despite the severe toxicity profiles. Their preliminary findings contradict the preclinical evidence from *in vitro* studies that showed

a lack of synergism with concurrent exposure to topoisomerase I and II inhibitors.

Platinum Agents and Amrubicin

We have identified five studies of AMR and platinum agents. The combination of first three studies was AMR and CDDP, and that of the last two studies was AMR and carboplatin (CBDCA). The first study, reported by our group, was a phase I study of AMR and CDDP in patients with previously untreated advanced NSCLC.[74] AMR was administered on days 1–3, and CDDP was administered at a fixed dose of 80 mg/m^2 on days 1, every 3 weeks. The MTD and recommended dose (RD) for AMR were determined to be at 30 mg/m^2. The second was a phase I study of AMR and CDDP in patients with previously treated NSCLC.[75] AMR was administered on days 1–3, and CDDP was administered at a fixed dose of 20 mg/m^2 on days 1–3, every 3 or 4 weeks. The MTD was determined to be at 30 mg/m^2 for AMR . The recommended dose was determined to be 25 mg/m^2 for AMR. The third was a phase I–II study of AMR and CDDP in previously untreated patients with ED-SCLC.[76] AMR was administered on days 1–3 and CDDP on day 1, every 3 weeks. The MTD was determined to be at 45 mg/m^2 for AMR and 60 mg/m^2 for CDDP. The RD was determined to be 40 mg/m^2 for AMR and 60 mg/m^2 for CDDP. The RR at the recommended dose was 87.8% (36/41 patients). The MST was 13.6 months and the 1-year survival rate was 56.1%. Grade 3/4 neutropenia and leukopenia occurred in 95.1% and 65.9% of patients, respectively. The forth was a phase I trial of AMR and CBDCA in previously untreated patients with ED-SCLC.[77] AMR and CBDCA were administered by intravenous infusion on days 1, 2, and 3, and on day 1, respectively. The MTDs of AMR and CBDCA were determined to be 40 mg/m^2 and the AUC was 5. A dose of 35 mg/m^2 AMR and CBDCA at AUC 5 was recommended in this regimen. The DLTs included neutropenia, leukopenia, thrombocytopenia, febrile neutropenia, and liver dysfunction. Evaluation of the responses revealed two patients with CR, nine with PR (RR 73%), and the MST was 13.6 months. The fifth was a phase I trial of AMR combined with CBDCA for elderly patients with SCLC,[70] and is described in the "Amrubicin Therapy for Elderly SCLC Patients" section.

In our pharmacological study, we established the relationships between AMR-OH and hematological toxicity during treatment with AMR alone, as well as during co-administration with CDDP, using a sigmoid E_{max} model for pharmacodynamic analysis.[60] The sigmoid curve for co-administration with CDDP was shifted to the left compared with that for AMR alone. This shift may indicate that patients treated with AMR and CDDP developed neutropenia more often than would be expected if they were treated with AMR alone. This mild additive effect in hematological toxicity is in agreement with clinical observations noted in many previous reports: patients receiving combined treatment with AMR and CDDP experienced more profound myelotoxicity than those treated with AMR alone, and the dose of AMR for combined treatment with CDDP was less than that used for AMR monotherapy.[74-76]

Phase III Studies

To our knowledge, AMR is currently undergoing phase III clinical studies in one monotherapy trial and two double combination regimen trials. The monotherapy trial involves patients with SCLC, after failure of first-line chemotherapy, comparing AMR with topotecan. The combination regimen trials comprise a randomized, multicenter study comparing CPT-11 with CDDP versus AMR with CDDP in the treatment of ED-SCLC; and a study of AMR with CDDP versus VP-16 with CDDP in ED-SCLC patients.

Amrubicin Therapy for Elderly SCLC Patients

In a first-line setting, AMR monotherapy for treating elderly and high-risk patients with SCLC has been reported.[78] A dose of 40 mg/m^2 on days 1–3 every 3 weeks was feasible, and had a favorable anticancer effect with an RR of 73%. Another phase I study used a combination therapy of AMR and CBDCA in previously untreated elderly SCLC patients.[70] DLTs were observed in all three patients at level 1 (AMR at 40 mg/m^2 and CBDCA at AUC 4.0) with grade 4 neutropenia or thrombocytopenia, or grade 3 diarrhea. The MTD of this combination therapy was AMR at 40 mg/m^2 and CBDCA at AUC 4.0, and the recommended dose for a phase II trial is AMR at 35 mg/m^2

and CBDCA at AUC 4.0. There are no reports of a second-line setting for AMR treatment of elderly patients with SCLC.

Future Approaches

Combination regimens that comprise agents with different mechanisms of action can result in synergistic antitumor activity and may overcome resistance to chemotherapy. In SCLC, combination chemotherapy generally yields higher overall RRs than does single agent therapy. However, care must be taken in the selection of agents to avoid overlapping toxicities that may adversely affect quality of life, especially in patients with extensive SCLC. To our knowledge, AMR is undergoing phase I or I–II clinical trials in combination regimens, including AMR plus TS-1 (tegafur, gimeracil and oteracil potassium), AMR plus nedaplatin, and AMR after concurrent VP-16 and CDDP plus accelerated hyperfractionated thoracic radiotherapy.

Unfortunately, it has been impossible in this review to cite all the references referring to the use of AMR in SCLC; likewise, we have not discussed the clinical trials of AMR performed in NSCLC patients. We have not discussed the downstream metabolites of AMR-OH. The detailed molecular mechanisms of how AMR induces apoptosis in cancer cells are unclear. Finally, most of the clinical trials with AMR have been performed in Japan: more trials conducted outside Japan are warranted.

Conclusions

It is clear that AMR, with its predictable and manageable toxicities, is one of the most attractive agents for the treatment of chemotherapy-sensitive and -refractory relapsed SCLC. Numerous studies are ongoing in an attempt to define the applicability of AMR as a single agent or in combination chemotherapy for patients with SCLC. These clinical trials, including phase III studies, will clarify the status of AMR in the treatment of SCLC.

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Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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Phase I Study of Topotecan and Cisplatin in Patients with Small Cell Lung Cancer

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Objective: A single-agent topotecan has an indication for the treatment of small cell lung cancer in Japan. Previous studies demonstrated that topotecan combined with a platinum agent could provide additional antitumor efficacy. This study was to find the recommended dose of topotecan in combination with cisplatin and preferred administration sequence in untreated patients with extensive disease small cell lung cancer for Phase II study.
Methods: Patients received topotecan as a 30 min infusion for 5 days in escalating doses (starting at 0.5 mg/m^2/day), and cisplatin at a fixed dose of 60 mg/m^2, 3 weeks cycle. This study employed the following stages: cisplatin was given before topotecan on day 1 to previously treated patients (Stage 1). After the maximum-tolerated dose level was achieved, the same schedule was applied for untreated patients (Stage 2). Subsequently, cisplatin was given after topotecan on day 5 to untreated patients (Stage 3). The recommended doses of cisplatin on day 1 and 5 schedules were estimated by considering results obtained from Stages 2 and 3, respectively.
Results: A total of 34 patients were enrolled. The maximum-tolerated doses in Stages 1–3 were estimated at 0.65, 0.65, and 1.4 mg/m^2, respectively. The recommended doses of cisplatin on day 1 and 5 schedules in untreated patients were determined at 0.65 and 1.0 mg/m^2, respectively. The major toxicity in this combination was hematological events.
Conclusions: For treatment-naive patients, the combined use of 0.65/60 mg/m^2 topotecan/cisplatin with cisplatin on day 1 schedule or 1.0/60 mg/m^2 topotecan/cisplatin with cisplatin on day 5 schedule is recommended for Phase II study.

Key words: small cell lung cancer – combination chemotherapy – topotecan – cisplatin

INTRODUCTION

Small cell lung cancer (SCLC) is often diagnosed at the extensive disease (ED) stage due to lesion location and rapid disease progression (1). Multiagent chemotherapy is the mainstay of treatment for SCLC, and combination regimens such as cisplatin + etoposide are being used as the standard therapy for ED cases (2). Topotecan, a topoisomerase-I inhibitor, has a favorable toxicity profile compared with most other agents that are active in SCLC. Topotecan has a well-characterized and predictable hematologic toxicity profile that includes neutropenia, which is manageable, short-lived and reversible. The non-hematologic effects of topotecan are generally mild and include manageable gastrointestinal toxicities (3). A single-agent topotecan showed significant activity in SCLC, particularly in patients sensitive to prior chemotherapy; therefore, the incorporation of topotecan in combination chemotherapy regimens for the future treatment of SCLC was warranted (3). Although single-agent topotecan has already an indication for the treatment of SCLC in Japan, previous preclinical and clinical studies have demonstrated that the combination of topotecan with a

platinum agent, such as cisplatin, could provide additional antitumor efficacy (4–9). In addition, one study addressed the impact of cisplatin scheduling and showed that the sequence of cisplatin before topotecan induced significantly worse hematological toxicity than the alternate sequence (10). To improve the therapeutic effect of this combination, the granulocyte colony-stimulating factor (G-CSF) was employed as concomitant therapy in our study.

Thus, we designed the present study to evaluate both administration sequences. The prime objective of the study was to determine the recommended dosage for a subsequent Phase II study from the estimation of maximum-tolerated dose (MTD) of topotecan in combination with 60 mg/m^2 cisplatin on day 1 or 5 in previously untreated patients with SCLC.

METHODS

Eligibility

Written informed consent was obtained from all patients prior to treatment. The protocol and informed consent procedures were reviewed and approved by the Institutional Review Board of each participating institute. Eligibility criteria were as follows: histologically or cytologically proven SCLC; 20–74 years old; previously treated with single-regimen chemo- and/or radiotherapy, or previously untreated patients with ED; no prior treatment with biological response modifiers within 2 weeks; adequate organic function (hemoglobin level $\geq$9.5 g/dl, leukocyte count of 4000–12 000/mm^3, neutrophil cell count $\geq$2000/mm^3, platelet count $\geq$100 000/mm^3, aspartate aminotransferase and alanine aminotransferase levels $<$2.5 times the upper limit of normal, total bilirubin value $<$1.5 mg/dl, serum creatinine below the upper limit of normal, partial pressure of arterial oxygen $\geq$60 mmHg); performance status of 0–1 on the Eastern Cooperative Oncology Group scale; a life expectancy of at least 3 months; and hospitalized patients.

Exclusion criteria included the following: serious infection or other serious concurrent disease; massive pleural effusion or ascites; interstitial pneumonia or pulmonary fibrosis; symptomatic central nervous system metastasis; concomitant malignancies; patients who received bone marrow or peripheral blood stem cell transplantation; a past history of drug allergy; actual or potential pregnancy, breast-feeding status or the intention to become pregnant in the near future; poorly controlled diabetes; previously treated with topotecan; or any other condition that was considered to make the patient ineligible for this study by the investigator.

Treatment Plan and Dose Escalation

This study employed the following three stages: Stage 1, cisplatin administration on day 1 followed by topotecan on days 1–5 to previously treated patients; Stage 2, on the same schedule as Stage 1 to previously untreated patients; and Stage 3, topotecan administration on days 1–5 followed by cisplatin on day 5 to previously untreated patients. The dose level of cisplatin was fixed at 60 mg/m^2 and the dose of topotecan was increased from 0.5 mg/m^2 followed by 0.65, 0.8, 1.0, 1.2 and 1.4 mg/m^2. The stage was moved up to the next after the MTD level was achieved, and the MTD was used as the starting dose in the next stage. Topotecan was administrated by 30 min intravenous infusion for 5 consecutive days. Cisplatin dissolved in 500–1000 ml of physiological saline was infused intravenously over 2 h either on day 1 (prior to topotecan) or day 5 (subsequent to topotecan). Immediately before and after each administration of cisplatin, hydration consisting of 1000–2000 ml of fluid infusion was given intravenously over 4 h. Treatment was repeated every 3 weeks and the next cycle could be expanded to a maximum of 35 days. At all dose levels of all stages, the prophylactic use of G-CSF was initiated from the next day of last administration of topotecan. G-CSF was administered till the recovery of leukocyte or neutrophil after nadir (recovery as either leukocyte count reached 10 000/mm^3 or neutrophil count reached 5000/mm^3).

In the present study, dose-limiting toxicity (DLT) was defined as follows: Grade 4 neutropenia lasting $\geq$4 days; Grade 3 or worse febrile neutropenia and thrombopenia ($<$20 000/mm^3); Grade 4 vasculitis, external auditory canal, fatigue, wound infection, ascites (non-malignant), constipation, central nervous system hemorrhage and hyponatremia; Grade 2 or worse middle ear/hearing, pneumonitis/pulmonary infiltrates and pulmonary fibrosis; and Grade 3 or worse other non-hematological toxicities excepting weight loss, syndrome of inappropriate antidiuretic hormone, anorexia, dyspepsia/heartburn, nausea, vomiting, incontinence and urinary frequency/urgency.

To assess the topotecan dose increase, three patients were enrolled at each dose level and the dose was increased to the next level if none of the patients displayed any DLT. If all the three patients showed DLT, then this dose level was defined as the MTD. If one of the three patients had DLT, an additional three patients were treated at the same level; if none/one of the three additional patients had DLT, the dose was increased to the next level. When two or more of the three additional patients had DLT, this dose level was defined as the MTD. If all the three additional patients showed DLT, the number of additional patients was determined by reference to results of estimation of the dose–response curve with a continual reassessment method. The recommended dose (RD) for Phase II study was determined to reflect the appearance of toxicity and antitumor effect as the drug dose less than the MTD.

Assessment of Treatment

Toxicities were assessed according to the US National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 2.0. The severity of other events not listed in the

NCI-CTC was graded as follows: Grade 1, slight; Grade 2, moderate; Grade 3, severe; and Grade 4, life threatening. During the study, complete blood cell counts and biochemistry tests were repeated at least twice weekly, whereas other investigations were repeated as needed to evaluate marker lesions. Response was evaluated according to the modified World Health Organization (WHO) criteria (11).

Table 1. Patient characteristics

	Stage 1	Stage 2	Stage 3
Cisplatin injection	On day 1	On day 1	On day 5
Prior treatment	(+)[a]	(−)	(−)
No. of patients	9	6	19
Gender			
Male	9	6	16
Female	0	0	3
Age			
Median	59	57	65
Range	23–70	50–74	48–74
Performance status (ECOG)			
0	5	1	5
1	4	5	14
Clinical stage			
I	1	0	0
III	5	0	3
IV	3	6	16

ECOG, Eastern Cooperative Oncology Group.
[a]Cases previously treated with single-regimen chemo- and/or radiotherapy.

RESULTS

Estimation of MTD

Between March 2000 and February 2005, 34 patients were enrolled in this study and all of them received chemotherapy. The characteristics of these patients are shown in Table 1 and the occurrence of DLTs is shown in Table 2.

Stage 1: Cisplatin on Day 1 and Topotecan on Days 1–5, for Previously Treated Patients

No DLT occurred at the first cycle in three patients who received a topotecan dose level of 0.5 mg/m^2, and the dose of topotecan was increased to 0.65 mg/m^2. Since one of the three patients displayed a DLT of thrombocytopenia (<20 000/mm^3), another three patients were treated at the same dose. One of the additional three patients indicated Grade 4 neutropenia lasting 4 days or more as DLTs. No more increase in dose in this stage was, however, decided by Extramural Evaluation Committee (EEC) since in three out of six cases indicated DLTs at the second cycle, although the DLTs were observed in two out of six cases in the first cycle. The MTD of this stage was estimated as 0.65 mg/m^2.

Stage 2: Cisplatin on Day 1 and Topotecan on Days 1–5, for Untreated Patients

The starting dose of topotecan in Stage 2 was 0.65 mg/m^2 based on Stage 1 results. As one of three patients showed Grade 4 neutropenia lasting 4 days or more as DLTs, additional three patients were treated at the same dose. One out of the three cases indicated thrombocytopenia (<20 000/mm^3) and Grade 4 neutropenia lasting 4 days or more as DLTs. Although two out of the six cases indicated DLTs at the first cycle, three of the six cases indicated thrombocytopenia (<20 000/mm^3) only or thrombocytopenia (<20 000/

Table 2. DLTs during the first cycle or other cycles at different dose levels

	Stage 1		Stage 2	Stage 3				
Topotecan (mg/m^2)	0.5[a]	0.65[a]	0.65[b]	0.65[b]	0.8[b]	1[b]	1.2[b]	1.4[b]
No. of assessable patients	3	6	6	4	6	3	3	3
Toxic effects with first cycle/other cycles								
Grade 4 neutropenia ≥4 days	0/0	1/0	1/0	0/0	0/0	0/0	0/0	1/0
Thrombocytopenia (<20 000/mm^3)	0/0	1/0	0/1	0/0	0/0	0/0	0/0	0/0
Grade 4 neutropenia ≥4 days and thrombocytopenia (<20 000/mm^3)	0/0	0/0	1/2	0/0	0/0	0/0	0/0	0/0
Grade3 non-hematological toxicity[c]	0/0	0/0	0/0	0/0	1/0	0/0	0/0	0/0
Gait disturbance	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0
Atrial fibrillation	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0

[a]Prior therapy.
[b]Therapy naive.
[c]Intravenous antibiotic injection for infection.

Table 3. Grade 3/4 toxicities in 85 cycles

	Stage 1		Stage 2	Stage 3				Overall
Topotecan (mg/m^2)	0.5	0.65	0.65	0.65	0.8	1.0	1.2	1.4	
No. of patients	3	6	6	4	6	3	3	3	34
No. of cycles	6	12	23	6	11	9	11	7	85
Mean of cycles	2	2	3.8	1.5	1.8	3	3.7	2.3	
Hematological toxicities									
Anemia	0 (0)[a]	3 (1)	4 (0)	0 (0)	0 (0)	2 (0)	1 (0)	1 (0)	32.4%
Leukopenia	1 (1)	4 (4)	6 (5)	0 (0)	0 (0)	1 (1)	0 (0)	1 (1)	38.2%
Neutropenia	2 (2)	4 (4)	6 (6)	0 (0)	0 (0)	1 (1)	1 (1)	1 (1)	44.1%
Thrombocytopenia	0 (0)	3 (2)	5 (3)	0 (0)	0 (0)	1 (0)	1 (1)	1 (1)	41.2%
Non-hematological toxicities									
Nausea	0 (0)	1 (1)	3 (2)	0 (0)	1 (1)	0 (0)	0 (0)	1 (1)	17.6%
Vomiting	0 (0)	1 (1)	2 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8.8%
Anorexia	0 (0)	1 (1)	3 (2)	0 (0)	1 (0)	0 (0)	0 (0)	1 (1)	17.6%
Interference with daily activity	0 (0)	1 (1)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	1 (1)	8.8%
Infection febrile	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2.9%
Increased amylase	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2.9%

Grade by the National Cancer Institute Common Toxicity Criteria (NCI-CTC).
[a]No. of patients in the first cycle are given in parentheses.

mm^3) and Grade 4 neutropenia lasting 4 days or more after the second cycle or later. EEC decided that no more proceed at this stage. Topotecan 0.65 mg/m^2 was estimated as the MTD of Stage 2, same with Stage 1.

Stage 3: Topotecan on Days 1–5 and Cisplatin on Day 5, for Untreated Patients

The first four patients, including one case who was decided as not evaluable from infection due to retaining needle, were treated with 0.65 mg/m^2 of topotecan based on the results obtained in Stage 2, and no DLT appeared. At the next dose level of 0.8 mg/m^2, one of three patients experienced DLTs, Grade 3 of infection as intravenous antibiotic injection. Then, additional three patients were enrolled at the same dose level, and none of the additional three patients had DLT. The dose of topotecan was increased as 1.0, 1.2 and 1.4 mg/m^2 in the protocol sequence. Three patients given 1.0 mg/m^2 and three patients given 1.2 mg/m^2 tolerated their dose level without DLT. Of the three patients given 1.4 mg/m^2, one patient developed Grade 4 neutropenia lasting 4 days. Following the hematological symptom, this patient also experienced Grade 3 gait disturbance. Furthermore, one case indicated an atrial fibrillation on day 3, although the relation between atrial fibrillation and topotecan was not clearly evidenced. Dose escalation was terminated at 1.4 mg/m^2; thus, we estimated the MTD of topotecan in this stage at 1.4 mg/m^2.

Gait disturbance occurred on day 10 of the first cycle following Grade 4 neutropenia. Twenty days later, in this patient, a cerebral infarction around the right lateral ventricle was observed by head magnetic resonance imaging diagnosis. This symptom has taken the medical history of cerebral hemorrhage without aftereffect and complications of hyperlipidemia and hyperuricemia into account. Then, this adverse event was observed for recovery tendency. Atrial fibrillation in a patient, who had a history of slight supraventicular arrhythmia without concomitant medication (not conflicted to exclusion criteria), noted at 1.4 mg/m^2 topotecan dose level, appeared just after completion of topotecan administration on day 3 in the first cycle and disappeared by oral anti-arrhythmic agent on the day 4 of the first cycle.

Toxicities

All 34 patients of 85 cycles were fully assessable for toxicity. Grade 3/4 toxicities during the overall cycles are summarized in Table 3. The most common hematological toxicity was neutropenia, followed by thrombocytopenia, leukopenia and anemia. On total comparison between the first cycle and overall cycles in hematological toxicity, at 0.65 mg/m^2 of Stage 1, 0.65 mg/m^2 of Stage 2 and 1.0 mg/m^2 of Stage 3, the occurrences of anemia and thrombocytopenia were increased. Leukopenia cases in 0.65 mg/m^2 of Stage 2 and anemia cases in over 1.2 mg/m^2 of Stage 3 were also increased. Average administration cycles in each dose

Table 4. Response to treatment

	Stage 1		Stage 2	Stage 3				
Cisplatin injection (fixed at 60 mg/m^2)	On day 1		On day 1	On day 5				
Prior treatment	(+)		(−)	(−)				
Topotecan (mg/m^2)	0.5	0.65	0.65	0.65	0.8	1.0	1.2	1.4
No. of patients	3	6	6	4	6	3	3	3
CR	0	0	0	0	0	0	0	0
PR	0	2	5	1	4	2	3	2
NC	2	3	0	0	0	0	0	0
PD	1	1	1	1	1	0	0	0
NE	0	0	0	2	1	1	0	1

CR, complete response; PR, partial response; NC, no change; PD, progressive disease; NE, not evaluable.

level in each stage were less than two cycles in 0.5 and 0.65 mg/m^2 of Stage 1 and 0.65 and 0.8 mg/m^2 of Stage 3, and over two cycles in other doses of Stages 2 and 3.

Principal non-hematological toxicities were observed in six cases of nausea and anorexia, three cases of vomiting and interference with daily activity and one case of infection febrile and increased amylase, excluded as DLT events.

Clinical Response

Clinical response is shown in Table 4. Twelve (63%) of 19 patients in Stage 3 yielded partial response (PR), whereas out of nine patients administered 1 mg/m^2 and more than 1.0 mg/m^2, seven (78%) showed PR.

Recommended Dose

The MTD of topotecan on days 1–5 for therapy-naive patients in combination with cisplatin on day 1 administration was estimated as 0.65 mg/m^2. The RD for Phase II was decided as 0.65 mg/m^2 by considering clinical response and toxicity. The MTD of topotecan in the case of cisplatin on day 5 administration was estimated as 1.4 mg/m^2. The DLTs of this dosage level were atrial fibrillation and gait disturbance in non-hematological toxicity. Since unexpected/serious non-hematological adverse events were observed at topotecan 1.4 mg/m^2 dose level, the RD was tentatively considered at 1.2 mg/m^2, in which dose level, no DLT cases were observed. However, after the first course of 1.0 mg/m^2 dose level, frequencies of Grade 3/4 anemia and thrombocytopenia, as hematological toxicities, indicated increased tendency. Thus, the RD for Phase II was decided at 1.0 mg/m^2, to secure adequate safety.

DISCUSSION

The combination of topotecan and cisplatin has been investigated in several studies. Boabang et al. (5) reported an *in vitro* study result indicating synergistic antitumor activity between topotecan and cisplatin presumably due to the inhibition of DNA repair mechanisms. Since this drug is categorized as an inhibitor against topoisomerase-I, a previous administration of a DNA-injuring drug seems to be useful (12). An *in vitro* combination effect with cisplatin to topotecan was, however, recognized either pre- or post-administration for topotecan. Compared with day 5 administration of cisplatin, day 1 administration indicated an increase in hematotoxicity from the pharmacokinetic mechanism caused by renal dysfunction suspected as subclinical renal tubular damage (10). From this consideration, a Phase I clinical study in therapy-naive SCLC patients aiming at RD finding was planned from the safety and efficacy of cisplatin day 1 and 5 administration schedules, under the G-CSF concomitant use for the prevention of leukopenia/neutropenia.

The MTD of topotecan in this study was 0.65 mg/m^2 for cisplatin day 1 administration schedule and 1.4 mg/m^2 for cisplatin day 5 administration schedule. The DLTs which lead these MTDs were hematological toxicities. At the first course, neutropenia was observed at two of six in the cisplatin day 1 administration group and one of three in the day 5 administration scheduled group. One out of six patients in the cisplatin day 1 group also experienced thrombocytopenia. As for neutropenia, the incidence was similar to the DLT in topotecan monotherapy Phase I (7,13,14). In combination therapy of cisplatin and topotecan, the DLT of thrombocytopenia was reported associated with neutropenia (7,15). In this study, toxicity data on the second and further courses were also evaluated. In this evaluation, no discrepancy was found between the first course and further courses, which established the DLT in this study as neutropenia and thrombocytopenia.

The non-hematological DLTs were gait disturbance and atrial fibrillation, which were observed in the cisplatin day 5 administration group at 1.4 mg/m^2 of topotecan. No non-hematological DLT was observed in the cisplatin day 1 administration group. Grade 3 non-hematological toxicities of nausea, vomiting, anorexia, fatigue and interference with daily activity were observed as similar to other studies such as topotecan monotherapy and cisplatin combination in which Grade 3/4 non-hematological toxicities of nausea, vomiting, anorexia, fatigue and so on were recorded (7,13,15). These DLTs seem not to be this drug specified from clinical observation on occurrence and progress. Furthermore, no occurrence of Grade 3/4 diarrhea was observed, as different from the similar drug of irinotecan (16).

The MTDs of Stages 1 and 2 were estimated by taking not only the DLTs during the first cycle but hematological toxicity after the second cycle or further cycles into account. The RD for cisplatin on day 1 schedule was estimated from

the MTD of 0.65 mg/m^2 derived from consideration on incidence increment of Grade 3/4 hematological toxicities in all cycles, although the DLT observations were seen in two of six cases in the first cycle. On the other hand, cisplatin on day 5 schedule, MTD was estimated as 1.4 mg/m^2 from the observation of DLT in two of three cases at a 1.4 mg/m^2 dose level. As for RD for Phase II, 1.2 to 1.0 mg/m^2 was recommended since hematological toxicity occurrence situations were almost equivalent between 1.2 and 1.0 mg/m^2 dose levels, then for initial dose was recommended as 1.0 mg/m^2 taking safety consideration into account, but increment to 1.2 mg/m^2 was to be allowed from the second cycle based on the occurrence situation of hematological toxicity.

Overseas, the RD for topotecan monotherapy Phase II in therapy-naive SCLC patients was 1.5 mg/m^2 (14). The RDs for cisplatin day 1 administration combination were topotecan 0.75 and cisplatin 75 mg/m^2, respectively (7,9). On the other hand, the RDs were reported as topotecan 1.50 or 1.25 mg/m^2 and cisplatin 50 mg/m^2, respectively, for therapy-naive SCLC in cisplatin day 5 administration schedule (15,17). In Japan, the RD of topotecan in monotherapy was 1.0 mg/m^2. From this study, the RD of topotecan was 65% decreased from monotherapy as 0.65 mg/m^2 in cisplatin day 1 administration schedule and there was no difference from monotherapy in cisplatin day 5 administration schedule. The topotecan RD ratio between cisplatin day 1 administration schedule and day 5 (0.65/1.0) was similar to overseas reports (0.75/1.25–1.50). Therefore, the RD of topotecan was one half of monotherapy in cisplatin day 1 administration schedule and same or 80% in cisplatin day 5 administration schedule, although cisplatin RD still remains uncertain. The reason for RD reduction in cisplatin day 1 schedule is suspected as the increment of hematotoxicity caused by topotecan clearance decrease by subclinical renal tubular damage (10).

The dose of combined cisplatin was employed at 60 mg/m^2 as same dose in the case of the similar drug of irinotecan combination (18). In this study, no DLT occurrence was observed in each three cases of topotecan 1.0 and 1.2 mg/m^2 groups and average administration course numbers were over 3, which indicated the tolerability of this combination. No significant difference in observed toxicities were found compared with monotherapy (19), and previously conducted topotecan and cisplatin combination clinical studies (7,9,15,17). These findings suggested the validity of the cisplatin dose of 60 mg/m^2 in this combination.

Since neutropenia is DLT in topotecan monotherapy (6), the prophylactic use of G-CSF was initiated on day 6 of topotecan administration in this study. As the major DLT of this study was, however, neutropenia, the administration timing of G-CSF in this study might not affect the incidence of DLT. According to Saltz et al. (19), although G-CSF administration did not affect topotecan dose increase, it was suggested that the administration of G-CSF supported prevention against secondary infection or recovery. From safety consideration, the G-CSF concomitant use in this combination seems to be practical.

In conclusion, the RDs of topotecan for 5 consecutive days in combination with 60 mg/m^2 cisplatin in a 3-week cycle were 0.65 mg/m^2 with cisplatin on day 1 with G-CSF and 1.0 mg/m^2, a maximum of 1.2 mg/m^2, with cisplatin on day 5 with G-CSF. Further Phase II study of this combination chemotherapy for advanced/metastasis SCLC as first-line therapy is ongoing.

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Conflict of interest statement

None declared.

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Innovator and generic cisplatin formulations: Comparison of renal toxicity

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To compare the incidence and degree of renal toxicity associated with innovator and generic cisplatin formulations, increase in the serum creatinine (CRN) levels (mg/dL) and incidence of grade 2–3 CRN elevation during the first and all cycles of chemotherapy were retrospectively evaluated in patients treated with innovator (group 1, $n = 296$) and generic (group 2, $n = 321$) cisplatin formulations. There were no differences in the sex, age, performance status or number of chemotherapy cycles between groups 1 and 2. The median increases in CRN levels during the first cycle were 0.20 mg/dL regardless of the sex or group. There was no difference in the incidence of grade 2–3 CRN elevation between groups 1 and 2 among female or male patients. The median increases in CRN levels during all cycles were 0.2 (0–1.0) and 0.3 (0–1.8) in the female patients of groups 1 and 2, respectively ($P = 0.68$), and 0.3 (0–2.1) and 0.5 (0–3.6) in the male patients of groups 1 and 2, respectively ($P < 0.001$). Grade 2–3 CRN elevation was observed in 18.1% and 24.7% of the female patients in groups 1 and 2, respectively ($P = 0.33$), and 9.4% and 20.9% of the male patients in groups 1 and 2, respectively ($P < 0.001$). Renal toxicity was slightly more severe in patients treated with the generic cisplatin formulation than in those treated with the innovator formulation, especially among the male patients. (*Cancer Sci* 2011; 102: 162–165)

Cisplatin, despite its severe toxicity, has been used in cancer chemotherapy for more than 30 years because of its significant therapeutic efficacy.[1] Although carboplatin, an analog of cisplatin with a milder toxicity profile, was also introduced for clinical use, randomized trials and meta-analyses showed that cisplatin-based chemotherapy was slightly superior to carboplatin-based chemotherapy in terms of the response rate and survival, at least in certain subgroups, without any significant increase in the incidence of severe toxicities among patients with germ cell tumor,[2] head and neck cancer,[3] and non-small-cell lung cancer.[4] In addition, cisplatin was shown to have a significant role in the treatment of bladder cancer, cervical cancer, esophageal cancer, ovarian cancer and small cell lung cancer, although carboplatin is being used increasingly in the treatment of some of these cancers as an alternative chemotherapeutic agent.[5] Thus, cisplatin still plays a pivotal role in the systemic treatment of a variety of solid tumors.

Renal toxicity is a major dose-limiting factor of cisplatin in most drug administration schedules.[6] Although the exact mechanism is unclear, the greatest concentration of platinum and widespread necrosis are reportedly observed in the proximal tubules of the kidney. This tubular impairment leads to a secondary reduction of renal blood flow and the glomerular filtration rate, potentiating the primary tubular damage. This vicious cycle causes delayed deterioration of renal function, as an increase in the serum creatinine (CRN) level typically appears 6–7 days after cisplatin administration in humans.[6] The standard prophylaxis for cisplatin nephrotoxicity is infusion of 1–4 L of normal saline on the day of cisplatin administration.[6] Although this vigorous hydration diminishes life-threatening renal toxicity, 7–40% of patients still develop a mild to moderate increase of serum CRN levels, which influences the subsequent cisplatin therapy.[7,8]

Generic substitutes serve as lower-cost alternatives to the more costly brand-name drugs for patients.[9] If it can be shown that a generic formulation is "essentially similar" in qualitative and quantitative composition to an innovator preparation, then the formulation can be marketed as "generic" without the need for expensive regulatory clinical trials. However, whether generic cisplatin formulations are truly therapeutically identical and interchangeable with innovator formulation of the drug has not yet been investigated. The objective of this study was to compare the severity of renal toxicity between an innovator cisplatin formulation and a generic substitute.

Patients and Methods

Patient selection. Patients were retrospectively selected for this study according to the following criteria: (i) a histological or cytological diagnosis of thoracic malignancy; (ii) no prior chemotherapy, except for a combination of uracil and ftorafur (UFT) as adjuvant chemotherapy after surgery; (iii) chemotherapy with a regimen that included 80 mg/m^2 of cisplatin; and (iv) receiving treatment as an inpatient at the National Cancer Center Hospital between November 2000 and March 2009. In this period, the innovator cisplatin formulation was administered between November 2000 and May 2004, and CISPLATIN for I.V. infusion (MARUKO), a generic cisplatin formulation, was administered thereafter. Patients with abnormal elevation of serum CRN before the start of chemotherapy were excluded from the current study.

Cisplatin administration. Cisplatin was administered at a dose of 80 mg/m^2 by intravenous infusion over 60–120 min on day 1 in combination with other chemotherapeutic agents, 40 g of mannitol and 3000 mL of hydration. On days 2–5, 2000 mL of intravenous infusion fluids were administered over 8 h. Antiemetic prophylaxis consisted of a $5HT_3$-antagonist and 16 mg of dexamethasone on day 1, followed by 8 mg of dexamethasone on days 2 and 3, 4 mg on day 4 and 2 mg on day 5. These treatments were repeated every 3–4 weeks. This sequence of administration was consistently maintained during the study period.

Data collection and statistical analyses. The patients' baseline characteristics, including age, sex and performance status (PS), pretreatment CRN level (CRNpre), chemotherapy regimen received, number of chemotherapy cycles administered and the maximum CRN level (CRNmax) during the first cycle and all cycles of chemotherapy were retrospectively obtained from medical charts. The patients' list was encoded and anonymized. The median CRNmax, median increase in the serum CRN levels (difference between CRNpre and CRNmax), and the Common

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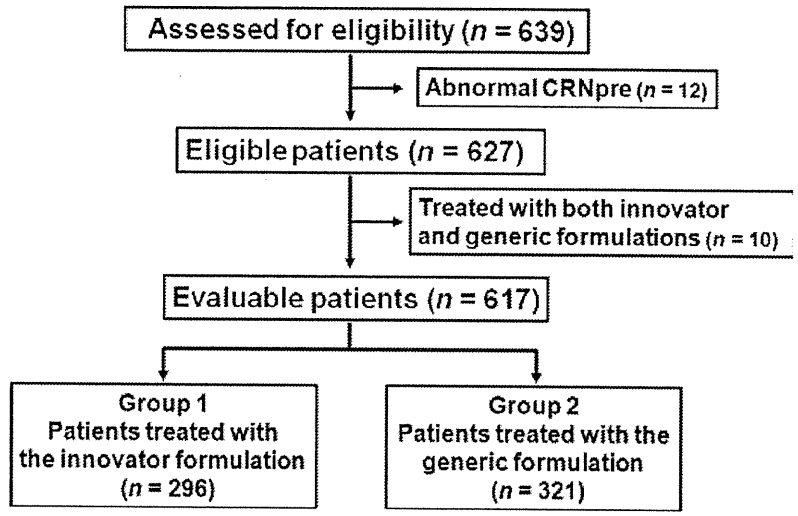

Fig. 1. Flow of patients. In the present study there was a total of 296 patients treated with an innovator cisplatin formulation (group 1) and 321 patients treated with a generic cisplatin formulation (group 2). CRNpre, pretreatment serum creatinine level.

Toxicity Criteria–Adverse Event (CTC-AE ver. 3.0) grades of the CRNmax were compared between patients treated with the innovator cisplatin formulation (group 1) and those treated with the generic formulation (group 2); these evaluations were performed for the entire study population, as well as separately for the female and male patients, because the normal range of the serum CRN level differs between the sexes. Mann–Whitney tests were used to evaluate continuous variables, and Chi-squared tests for categorical variables. The Dr SPSS II 11.0 for Windows software (SPSS Japan Inc., Tokyo, Japan) was used for the statistical analyses.

This study was approved by the president of the National Cancer Center Hospital, Tokyo, Japan. The institutional review board and ethical review committee decided to exempt this study from the usual review process because of its retrospective nature.

Results

Of the 639 patients assessed for eligibility in this study, 627 patients met the inclusion criteria; 12 patients were excluded because of abnormal CRNpre levels. An additional 10 patients were excluded because they were treated with the innovator cisplatin formulation in the first cycle of chemotherapy, but with the generic formulation in subsequent cycles. Thus, a total of 617 patients were included as the subjects of this study. Of these, 296 patients were treated with the innovator cisplatin formulation (group 1) and 321 were treated with the generic formulation (group 2) (Fig. 1). The median age of the patients was 60 years in both groups, and female patients accounted for 24% of all patients (Table 1). There were no meaningful differences in the PS, CRNpre levels, height, weight or number of chemotherapy cycles between the groups.

The median (range) CRNmax levels during the first cycle of chemotherapy were 0.7 (0.5–1.8) mg/dL and 0.7 (0.5–1.6) mg/dL in the female patients of group 1 and group 2, respectively ($P = 0.25$), while they were 1.0 (0.5–4.5) mg/dL and 1.1 (0.6–4.2) mg/dL, respectively, in the male patients of group 1 and group 2, respectively ($P = 0.016$). These differences were even more pronounced when the CRNmax levels during all cycles of chemotherapy were evaluated. The median (range) CRNmax level in the female patients did not differ between groups 1 and 2 (0.8 [0.5–1.8] mg/dL *vs* 0.9 [0.6–2.5] mg/dL, $P = 0.22$), whereas that in the male patients was higher in group 2 than in group 1 (1.1 [0.5–4.5] mg/dL *vs* 1.3 [0.7–4.2] mg/dL, $P < 0.001$).

Table 1. Patient characteristics

Characteristics	Group 1† (n = 296)	Group 2‡ (n = 321)	P-value
	N (%)	N (%)	
Sex			
Females	72 (24)	77 (24)	0.93
Males	224 (76)	244 (76)	
Age (years)			
Median (range)	59.5 (18–77)	60.0 (18–75)	0.85
Height (cm)			
Median (range)	164 (146–181)	165 (143–189)	0.03
Weight (kg)			
Median (range)	60.6 (35.3–102)	60.3 (33.9–106)	0.76
PS			
0–1	294 (99)	317 (99)	0.69
2–3	2 (1)	4 (1)	
CRNpre			
Median (range)	0.7 (0.3–1.1)	0.7 (0.3–1.1)	0.17
No. cycles			
1–2	121 (41)	123 (38)	0.56
3–5	175 (59)	198 (62)	
Median (range)	3 (1–5)	3 (1–4)	

†Patients treated with innovator formulation. ‡Patients treated with generic formulation. CRNpre, pretreatment serum creatinine level.

The increases in the serum CRN levels (differences between the CRNmax and CRNpre levels) during the first cycle of chemotherapy are summarized in Table 2. The median increase of the serum CRN levels did not differ between the female patients of groups 1 and 2, while it was higher in the male patients of group 2 than in those of group 1. However, the percentage of patients with a significant increase of serum CRN levels (0.7 mg/dL or higher) did not differ between groups 1 and 2 in the entire subject population, in the female patients alone, or in the male patients alone. The clinical significance of the increase in serum CRN levels was assessed using the CTC-AE grade of serum CRN. The CRN CTC-AE grades during the first cycle of chemotherapy did not differ between groups 1 and 2 in the entire subject population, in the females alone, or in the males alone (Table 2).

There was a definite increase in the serum CRN levels during all cycles of chemotherapy (Table 3). The median increase in the serum CRN levels did not differ between the female patients

Table 2. Increase in serum creatinine levels and toxicity grades during the first cycle of chemotherapy

	Group 1† (n = 296)	Group 2‡ (n = 321)	P-value
	N (%)	N (%)	
Increase in serum creatinine levels (mg/dL)			
Total			
0–0.3	242 (81.8)	238 (74.1)	0.070
0.4–0.6	35 (11.8)	51 (15.9)	
≥0.7	19 (6.4)	32 (10.0)	
Median (range)	0.2 (0–1.0)	0.2 (0–1.2)	0.054
Female			
0–0.3	64 (88.9)	66 (85.7)	0.76
0.4–0.6	5 (6.9)	8 (10.4)	
≥0.7	3 (4.2)	3 (3.9)	
Median (range)	0.2 (0–1.0)	0.2 (0–1.2)	0.90
Male			
0–0.3	178 (79.5)	172 (70.5)	0.070
0.4–0.6	30 (13.4)	43 (17.6)	
≥0.7	16 (7.1)	29 (11.9)	
Median (range)	0.2 (0–2.1)	0.2 (0–3.6)	0.027
CTC-AE grade			
Total			
0	211 (71.3)	208 (64.8)	0.20
1	69 (23.3)	87 (27.1)	
2–3	16 (5.4)	26 (8.1)	
Female			
0	43 (59.3)	40 (51.9)	0.60
1	23 (31.9)	28 (36.4)	
2–3	6 (8.3)	9 (11.7)	
Male			
0	168 (75.0)	168 (68.9)	0.29
1	46 (20.5)	59 (24.2)	
2–3	10 (4.5)	17 (7.0)	

†Patients treated with innovator formulation. ‡Patients treated with generic formulation. CTC-AE, Common Toxicity Criteria–Adverse Event ver. 3.0.

Table 3. Increase in serum creatinine levels and toxicity grades during all cycles of chemotherapy

	Group 1† (n = 296)	Group 2‡ (n = 321)	P-value
	N (%)	N (%)	
Increase in serum creatinine levels (mg/dL)			
Total			
0–0.3	177 (59.8)	146 (45.5)	<0.001
0.4–0.6	71 (24.0)	85 (26.5)	
≥0.7	48 (16.2)	90 (28.0)	
Median (range)	0.3 (0–2.1)	0.4 (0–3.6)	<0.001
Female			
0–0.3	49 (68.1)	52 (67.5)	0.99
0.4–0.6	15 (20.8)	16 (20.8)	
≥0.7	8 (11.1)	9 (11.7)	
Median (range)	0.2 (0–1.0)	0.3 (0–1.8)	0.68
Male			
0–0.3	128 (57.1)	94 (38.5)	<0.001
0.4–0.6	56 (25.0)	69 (28.3)	
≥0.7	40 (17.9)	81 (33.2)	
Median (range)	0.3 (0–2.1)	0.5 (0–3.6)	<0.001
CTC-AE grade			
Total			
0	160 (54.1)	122 (38.0)	<0.001
1	102 (34.5)	129 (40.2)	
2–3	34 (11.5)	70 (21.8)	
Female			
0	30 (41.7)	24 (31.2)	0.44
1	29 (40.3)	34 (44.2)	
2–3	13 (18.1)	19 (24.7)	
Male			
0	130 (58.0)	98 (40.2)	<0.001
1	73 (32.6)	95 (38.9)	
2–3	21 (9.4)	51 (20.9)	

†Patients treated with innovator formulation. ‡Patients treated with generic formulation. CTC-AE, Common Toxicity Criteria–Adverse Event ver. 3.0.

of groups 1 and 2, while it was higher in the male patients of group 2 than in those of group 1. The percentage of patients with an increase in CRN levels of ≥0.7 mg/dL did not differ between the female patients of groups 1 and 2, but was higher in the male patients of group 2 than in those of group 1 (33.2% *vs* 17.9%, $P < 0.001$). The percentage of patients with grade 2–3 CRN CTC-AE in the male patients was 20.9% in group 2 and 9.4% in group 1 ($P < 0.001$), although no significant difference was noted between the female patients of groups 1 and 2 (Table 3).

Since we unexpectedly found a distinct trend of increase in the CRN levels in the female and male patients, we studied the gender difference (Table 4). The increase in CRN levels was higher in the male than female patients, while the CRN CTC-AE grades were more severe in the female than male patients.

Discussion

This study showed that the renal toxicity was slightly more severe in the patients who were treated with the generic cisplatin formulation than in those treated with the innovator formulation, especially among male patients. This result was not attributable to biased prognostic factors for cisplatin-induced renal toxicity, including age, PS, dose of cisplatin or the number of chemotherapy cycles pointed out previously,[7,8] because these variables were distributed equally between patients who were treated with the generic and innovator cisplatin formulations. Higher CTC-AE grades as well as increased serum CRN levels during chemotherapy were observed in patients treated with the generic cisplatin formulation, and therefore this renal toxicity can be as severe as it influences subsequent therapies of the patients.

The generic drug is exactly the same as the innovator drug in its basic composition and property, including cisplatin content, solvent and pH and osmotic pressure of the solution, but the additives to stabilize the solution may not be identical. Thus, this small difference is considered to result in the increased renal toxicity of the generic drug. Unlike the basic composition and property of drugs, the influence of additives can not be easily examined. It is much simpler to use the same additives in the manufacturing process of generic drugs.

We never expected that the association between increased renal toxicity and administration of the generic cisplatin formulation would be more evident in male patients, and this result prompted us to compare the renal toxicity between the sexes. A rise in the serum CRN levels during chemotherapy was more frequent in male patients, while the CTC-AE grades were more severe in the female than male patients. This is probably because men generally have a larger and more muscular physique that leads to higher CRN production and a higher upper limit of the normal range of the serum CRN level in men than in women. In previous studies, a rise in serum CRN during cisplatin-containing chemotherapy was found to be slightly more frequent in female patients, but the difference was only mild and of no clinical significance. Thus, there seems to be only a small difference, if any, in the responses to nephrotoxic agents between the sexes, and males are unlikely to be more vulnerable to cisplatin. Another possible explanation for the current results might be the

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