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ORIGINAL ARTICLE

Phase I and pharmacologic study of irinotecan and amrubicin in advanced non-small cell lung cancer

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Abstract
Purpose We conducted a Phase I trial of irinotecan (CPT-11), a topoisomerase I inhibitor, combined with amrubicin, a topoisomerase II inhibitor. The aim was to determine the maximum tolerated dose (MTD) of amrubicin combined with a fixed dose of CPT-11 as well as the dose-limiting toxicities (DLT) of this combination in patients with advanced non-small cell lung cancer.
Patients and methods Eleven patients with stage IIIB or IV disease were treated at 3-week intervals with amrubicin (5-min intravenous injection on days 1–3) plus 60 mg/m^2 of CPT-11 (90-min intravenous infusion on days 1 and 8). The starting dose of amrubicin was 25 mg/m^2, and it was escalated in 5 mg/m^2 increments until the maximum tolerated dose was reached.
Results The 30 mg/m^2 of amrubicin dose was one dose level above the MTD, since three of the five patients experienced DLT during the first cycle of treatment at this dose level. Diarrhea and leukopenia were the DLT, while thrombocytopenia was only a moderate problem. Amrubicin did not affect the pharmacokinetics of CPT-11, SN-38 or SN-38 glucuronide. Except for one patient, the biliary index on day-1 correlated well with the percentage decrease of neutrophils in a sigmoid E_{max} model. There were five partial responses among 11 patients for an overall response rate of 45%.
Conclusion The combination of amrubicin and CPT-11 seems to be active against non-small cell lung cancer with acceptable toxicity. The recommended dose for Phase II studies is 60 mg/m^2 of CPT-11 (days 1 and 8) and 25 mg/m^2 of amrubicin (days 1–3) administered every 21 days.

Keywords CPT-11 · Amrubicin · Topoisomerase inhibitors · Pharmacokinetics · Biliary index

Introduction

Amrubicin, a totally synthetic 9-aminoanthracycline [9], is converted to an active metabolite, amrubicinol, through the reduction of its C-13 ketone group to a hydroxy group. The In vitro cytotoxic activity of amrubicinol is 18 to 220 times more potent than that of its parent compound, amrubicin [30]. Despite their similarity in chemical structure, amrubicin is rather different from doxorubicin in its mode of action [7]. Amrubicin and amrubicinol are inhibitors of DNA topoisomerase II. In preclinical studies, amrubicin showed more potent antitumor activity than doxorubin in several human tumor xenografts implanted in nude mice [17], and showed almost no cardiotoxicity [20, 26]. The response rates to amrubicin at 45 mg/m^2 on days 1–3 in chemotherapy-naive patients with stage III or IV non-small cell lung cancer (NSCLC) and extensive stage small cell lung cancer were 25 and 79%, respectively [5, 31].

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Irinotecan (CPT-11) is a water-soluble camptothecin derivative that inhibits topoisomerase I. CPT-11 has shown significant antitumor activity against various malignancies including lung cancer [2, 4, 14].

DNA topoisomerase I and II are functionally related and act in concert. Therefore, the combined use of topoisomerase I and II inhibitors is theoretically attractive because of the complementary functions of their targets [28].

Based on the different cellular targets (topoisomerase I for CPT-11 and topoisomerase II for amrubicin), the single-agent activity of amrubicin and CPT-11 against lung cancer, and the results of the preclinical studies showing the potentiation of single-agent activity with the coadministration of both drugs, a Phase I trial of this combination in patients with advanced NSCLC was conducted. The objectives of this Phase I study were to determine the maximum tolerated dose (MTD) of amrubicin and CPT-11; to detect and quantify the clinical toxicities of this combination; to investigate the pharmacokinetics of amrubicin, its active metabolite, amrubicinol, CPT-11 and its active metabolite, SN-38, SN-38 glucuronide (SN-38G); and to obtain preliminary evidence of the therapeutic activity of this combination in patients with advanced NSCLC.

Patients and methods

Patient selection

Patients were enrolled in this study if they met the following criteria: a histologic or cytologic diagnosis of NSCLC; a stage IV disease, or stage IIIB disease that was not a candidate for curative radiation therapy; a measurable lesion; no prior chemotherapy; a performance status of 0 or 1 on the Eastern cooperative oncology group (ECOG) scale; a life expectancy of at least 3 months; adequate bone marrow function (leukocyte count $\geq$ 4,000/μl, platelet count $\geq$ 100,000/μl, and hemoglobin $\geq$ 10.0 g/dl), adequate hepatic function [aspartate amino-transferase (AST) and alanin amino-transferase (ALT) levels of $\leq$ 100 IU/l, and bilirubin $\leq$ 2.0 mg/dl ($\leq 2 \times$ the upper limit of normal)], adequate renal function (creatinine $\leq$ the upper limit of normal) and arterial oxygen partial pressure ($PaO_2 \geq 60$ torr); electrocardiogram findings within the normal range; between 20 and 69 years of age; written informed consent to the study. Patients were ineligible if they had serious infectious diseases or other severe complications (heart diseases, interstitial pneumonia, or uncontrollable diabetes); had watery diarrhea, paralytic ileus, or intestinal obstruction; had massive pleural or pericardial effusion, or ascitic fluid; had symptomatic brain metastases; had active concurrent malignancies; were lactating or pregnant women, or those willing to be pregnant; had a history of a drug allergy; had a history of acute myocardial infarction within the previous 12 months; had superior vena caval syndrome, which requires urgent radiotherapy; had other medical problems severe enough to prevent compliance with the protocol. The study was approved in advance by the Kitasato University Hospital Ethics Committee.

Drug administration

Treatment cycle was planned to be 21 days. All of the agents were from commercial suppliers. CPT-11 (Yakult Honsha Co., Ltd., Tokyo, Japan) was obtained as 5 ml vials containing 100 mg of the drug, and was diluted in 500 ml of normal saline for administration, then administered to the patient at a fixed dose of 60 mg/m^2 as a 90-min intravenous infusion on days 1 and 8. This dose was chosen because it has previously shown high efficacy in combination with cisplatin or etoposide. CPT-11 treatment on day-8 was postponed until day-15 if the leukocyte count was less than 3,000/μl, platelet count < 100,000/μl, fever > 38°C or Grade $\geq$ 1 diarrhea.

Amrubicin was supplied from Sumitomo Pharmaceuticals Co., Ltd. (Osaka, Japan) as a lyophilized light red powder containing 20 mg of amrubicin. The appropriate amount of amrubicin was dissolved in 20 ml of normal saline and administered intravenously as a 5-min infusion on days 1 to 3 just after the completion of the CPT-11 infusion.

Dosage, and dose escalation procedure

The study was designed to escalate the dose of amrubicin in increments of 5 mg/m^2 for successive patient cohorts until the MTD was reached. CPT-11 was administered at a fixed dose of 60 mg/m^2 intravenously over 90 min on days 1 and 8. The dose-limiting toxicities (DLT) were defined as: the absolute neutrophil count (ANC) < 500/μl or leukocyte count < 1,000/μl for $\geq$ 4 days; febrile neutropenia (fever > 38.5°C with ANC < 1,000/μl); platelet count < 10,000/μl; and non-hematologic toxicity (except for nausea) $\geq$ Grade 3. The toxicity forcing the delay in CPT-11 treatment on day 8 > 7 days, and the toxicity resulting in treatment delay > 14 days in the next cycle were also considered to be DLT. At least three patients were to be included at each dose level. Escalation to the next dose level was allowed if none of the three patients had DLT during the first cycle of treatment. If one of the three patients had DLT, then the level was expanded to six patients.

If < two out of the six patients experienced DLT, further dose escalation was allowed. If ≥ two out of the six patients experienced DLT, then that dose was greater than the maximum tolerated dose (MTD). The MTD was defined as the highest level at which one or fewer of six patients experienced DLT, but was immediately below a dose level at which two or more patients experienced DLT. A total of six patients were to be treated at the recommended dose level to gain further experience with the combination as well as to provide a preliminary assessment of antitumor activity. No intrapatient dose escalation was allowed in this trial. Since the probability of occurrence of DLT at any dose level was set to be 33%, the chance of over-escalation was 43.5% in this trial.

Patients whose condition stabilized or improved received at least a second course of treatment. Patients with obvious evidence of disease progression or those who experienced intolerable toxicity were removed from the study. If more than 4 weeks passed from the time of the last treatment before these criteria were satisfied, the patient was also removed from the study. Before the next course was started, the WBC count had to be 3,000/μl or higher, the platelet count had to be 100,000/μl or higher, diarrhea should have resolved completely, and any other toxicities (except for nausea) had to be ≤ Grade 2.

Evaluation

Tumors were staged based on a complete medical history and physical examination, routine chest radiography, bone scintiscanning, computed tomography (CT) of the chest and abdomen, whole-brain magnetic resonance imaging (MRI) or CT scan, and fiberoptic bronchoscopy. Staging was performed according to the tumor-node-metastasis system [18]. Prior to the first course of treatment, a complete blood count (including a differential white cell count and platelet count), biochemistry tests (renal function, hepatic function, and electrolytes), electrocardiogram, and urinalysis were performed. The complete blood count and biochemistry tests were repeated at least once a week after this initial evaluation, while the other investigations were repeated at least every 6 weeks to evaluate the target lesions. A complete blood count was repeated every day until recovery, when ANC < 500/μl, leukocyte count < 1,000/μl, or platelet count < 10,000/μl was observed. Adverse events were recorded and graded using the National Cancer Institute Common Toxicity Criteria, Version 2.0. The tumor response was classified in accordance with Response evaluation criteria in solid tumors (RECIST) [27]. The duration of the response was defined as the number of days from the documentation of the response to the detection of disease progression.

Pharmacokinetics

Heparinized blood samples (5 ml) for the pharmacokinetic study were obtained from the opposite arm of each patient before CPT-11 infusion, at the end of the infusion, and 1, 2, 4, 7, and 24 h after the completion of infusion on days 1 and 8 during the first treatment cycle. The blood was centrifuged immediately, and 2 ml of the plasma thus obtained was placed into a tube containing 2 ml of 0.146 *M* phosphate and stored at–20°C until analysis. Heparinized blood samples (3 ml) for the pharmacokinetic study of amrubicin were also obtained before and at 15 min and 1, 3, 8, and 24 h after completing the infusion on Days 1 and 3. The plasma levels of CPT-11, SN-38, and SN-38G were determined by the method of Kurita et al. [25] using the high-performance liquid chromatography (HPLC) method with a fully automated on-line solid-phase extraction system (Prospect; Spark Holland, Emmen, the Netherlands) as previously described. The quantification limit for CPT-11, SN-38, and SN-38G was 2 ng/ml. Linearity was confirmed up to 25,000 ng/ml for CPT-11 ($r = 0.999998$), 2,500 ng/ml for SN-38 ($r = 0.999998$), and 500 ng/ml for SN-38G ($r = 1.000000$) in the plasma. For amrubicin and amrubicinol, the concentrations in plasma were assayed as previously reported [21]. In brief, amrubicin and amrubicinol in the plasma (0.1 ml) were extracted with ethyl acetate under alkaline conditions. After centrifugation, the organic layer was evaporated at 40°C under a gentle stream of nitrogen. The residue was dissolved in 0.6 ml of methanol. The amrubicin and amrubicinol concentrations were determined using HPLC and mass spectrometry (HPLC-MS/MS). HPLC-MS/MS was performed using an API-300 (Applied Biosystems/MSD SCIEX, CA, USA) tandem mass spectrometer equipped with a turbo ionspray source in positive ion mode connected to an Alliance 2,690 Separations Module (Waters, MA, USA) chromatograph. The calibration range was 20–3,000 ng/ml for the analytes in 0.1 ml of plasma.

Pharmacokinetic analyses

The following noncompartmental pharmacokinetic parameters of amrubicin, amrubicinol, CPT-11, SN-38, and SN-38G were estimated using WinNonlin Version 4.1(Scientific Consultant, Apex, NC) [29]. C_{max} was the maximum drug concentration after the intravenous infusion. The terminal rate constant (K) was determined by log-linear regression analysis of the terminal phase of

the plasma concentration-time curves. The terminal half-lives ($t_{1/2}$) were calculated by the equation: $t_{1/2} = 0.693/K$. $AUC_{0\text{-}\infty}$ was calculated by the linear trapezoidal rule from time zero up to the last measurable data point with extrapolation to infinity. Clearance (CL) was calculated by dividing the dose received by the AUC. The volume of distribution at steady state (Vss) was calculated by the equation CL × [mean residence time (MRT)]. The biliary index (BI) was calculated as the $AUC_{CPT\text{-}11} \times AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ as previously described [6]. The percent change in ANC was calculated using the following equation: % change in ANC = ([pretreatment ANC−nadir ANC]/pretreatment ANC)×100 [8]. The relationship between the percent change in the ANC and BI was explored using a sigmoid maximum-effect model as follows: $\text{Effect}\ (\%) = 100 \times E_{max}(BI)^{\kappa} / [(BI_{50})^{\kappa} + BI^{\kappa}]$. Nonlinear least-squares regression performed with WinNonlin Version 4.1 was used to estimate the BI that produces 50% of the maximum effect (BI_{50}) and the sigmoidicity coefficient (κ). Other statistical analyses were performed using the chi-square test or Fisher's exact test, and $P < 0.05$ was considered to be statistically significant.

Results

Between July 2003 and November 2004, 11 patients participated in this trial. A profile of the patient population is given in Table 1. Six patients were women and five were men, and the median age was 59 years (range 46–64 years) with a median performance status of 0. Dosing information is shown in Table 2. In this study, a total of 37 courses of therapy were given. The number of treatment cycles administered per patient ranged from 1 to 6 (two cycles in four patients, three in two, four in three, five in one, and six in one patient). All treated patients were assessed for toxicity and response.

Table 1 Patient characteristics

Total no. of patients	11
Sex	
Male	5
Female	6
Age: Median (range)	59 years (46–64)
Performance status (ECOG)	
0	7
1	4
Stage	
IIIB	2
IV	9
Histology	
Adenocarcinoma	8
Squamous cell carcinoma	2
Large-cell carcinoma with neuroendocrine properties	1

Toxicities

DLT

First, three patients were enrolled at dose level 1 (amrubicin 25 mg/m^2 on days 1–3 and CPT-11 60 mg/m^2 on days 1 and 8). No DLT was observed during the first course of dose level 1. Grade 3 diarrhea, despite maximal antidiarrheal support with high-dose loperamide, was the DLT in the first cycle at the second dose level (amrubicin 30 mg/m^2 on days 1–3 and CPT-11 60 mg/m^2 on days 1 and 8) in one of three patients (Table 3). When this dose level was expanded to five patients, CPT-11 treatment on day-8 was withheld in another two patients because of sustained Grade 2 leukopenia on days 8–15. This CPT-11 treatment omission on day-8 also emerged as DLT. Dose level 2 was exceeded the MTD because three out of five patients developed DLT at this level. Further attempts to intensify the dose were not conducted. Three additional patients were then treated at the recommended dose level (dose level 1) to confirm its feasibility. Ultimately, one of six patients experienced DLT per protocol definition with CPT-11 treatment omission on day-8 because of sustained Grade 2 leukopenia in the first cycle.

Hematologic toxicity

Table 4 shows the worst toxicities experienced during the treatment. The most frequent toxicities were leukopenia and neutropenia. Although there was no Grade 4 neutropenia at dose level 1, short-lived Grade 4 neutropenia occurred in three (60%) of five patients

Table 2 Dose escalation scheme and treatment administered to patients receiving amrubicin and CPT-11

Dose (mg/m^2)					Delivered dose/planned dose of CPT-11 and AMR
Dose level	CPT-11 (days 1, 8)	Amrubicin (days 1–3)	No. of patients	Total no. of courses	
1	60	25	6	22	84 and 100%
2	60	30	5	15	93 and 100%

Table 3 Dose-limiting toxicities (DLT) during the first cycle at different dose levels

	Dose level	
	1	2
No. of patients	6	5
DLT		
Delay in CPT-11 treatment on day-8 for more than 7 days	1	2
Diarrhea	0	1
No. of patients who experienced DLT	1	3

at dose level 2 (Table 4). Seven episodes of Grade 3/4 neutropenia were reported during this trial. Two (29%) of seven episodes of Grade 3/4 neutropenia were asymptomatic, whereas one episode (14%) was associated with fever, and another (14%) was complicated with Grade 3 diarrhea. The median time to neutrophil nadir for nine patients experiencing neutropenia during the first course was 16 days, with the median time to recovery being 6 days. Other toxicities were less than Grade 3.

Non-hematologic toxicity

Although there was no Grade 3 or worse diarrhea at the first dose level, one (20%) occasion of Grade 3 or worse diarrhea was noted at the second dose level (Table 4). Furthermore, this episode was associated with asymptomatic Grade 4 neutropenia. At dose levels 1 and 2, the first course caused Grade 2 or worse diarrhea in 33 and 20% of the patients, respectively. Grade 3 or worse nausea and anorexia occurred in two patients treated at dose level 1.

Table 4 Worst toxicities at different dose levels

	Dose level 1 ($n = 6$)				Dose level 2 ($n = 5$)			
	Grade				Grade			
	0–1	2	3	4	0–1	2	3	4
Leukopenia	2	3	1	0	1	0	4	0
Neutropenia	3	0	3	0	0	1	1	3
Thrombocytopenia	6	0	0	0	4	1	0	0
Anemia	2	4	0	0	2	3	0	0
Nausea	3	1	2	0	4	1	0	0
Vomiting	4	2	0	0	5	0	0	0
Anorexia	3	1	2	0	3	2	0	0
General fatigue	6	0	0	0	5	0	0	0
Diarrhea	4	2	0	0	3	1	1	0
Abnormal liver function	6	0	0	0	5	0	0	0
Abnormal renal function	6	0	0	0	5	0	0	0
Rash	5	1	0	0	5	0	0	0
Constipation	3	3	0	0	5	0	0	0
Dizziness	6	0	0	0	5	0	0	0
Interstitial pneumonia	6	0	0	0	4	1	0	0
Alopecia	5	1	0	0	5	0	0	0
Stomatitis	6	0	0	0	5	0	0	0
Abdominal pain	6	0	0	0	5	0	0	0
Injection site reaction	5	1	0	0	4	1	0	0

Other non-hematologic toxicities of Grade 2 included nausea and vomiting, anorexia, skin rash, diarrhea, constipation, interstitial pneumonia, alopecia, and injection site reaction. No cardiotoxicity was observed during this trial.

There was no evidence of the cumulative toxicity of leukopenia, anemia, or alopecia in the subsequent courses at two dose levels. There were no treatment-related deaths during this trial.

Pharmacokinetics

Plasma samples for amrubicin were obtained from eight patients during their first course of treatment (days 1–3). Three patients were treated with 25 mg/m^2 of amrubicin, while five patients received 30 mg/m^2. The plasma concentration-time curves for the different doses of amrubicin are shown in Fig. 1, and the pharmacokinetic parameters derived from the plotted data are listed in Table 5. In accordance with the results obtained with amrubicin used as a single agent [19], there was no accumulation of amrubicin on days 1 and 3, whereas the AUC of amrubicinol on day-3 increased by 76.5% than that on day-1. The other pharmacokinetic results obtained here are also consistent with those obtained in the previous phase I trial [19]. There was little correlation between the dose of amrubicin and the plasma AUC value ($r = 0.06976$; $P = 0.8696$).

For CPT-11, SN-38, and SN-38G the pharmacokinetics were obtained in eight patients on day-1 (Fig. 2 and Table 6). The C_{max} of CPT-11 was attained around the end of the infusion, and the plasma disappearance was biphasic. SN-38 and SN-38G were rapidly formed from the parent compound, and the C_{max} was reached between 0 and 2 h after the completion of the CPT-11 infusion. The plasma concentrations of SN-38 and SN-38G decreased more slowly than that of CPT-11 (Fig. 2). The AUC of SN-38G was well related to the AUC of CPT-11 ($r = 0.86869$; $P = 0.0051$). In contrast, the AUC of SN-38 was poorly related to that of CPT-11 ($r = 0.51873$; $P = 0.1878$), because there were wide inter-individual differences in the kinetics of SN-38. There were no significant differences in pharmacokinetic parameters seen on days 1 and 8 of CPT-11 administration. There was no accumulation of CPT-11, SN-38, and SN-38G on days 1 and 8, suggesting no apparent drug interaction between amrubicin and CPT-11.

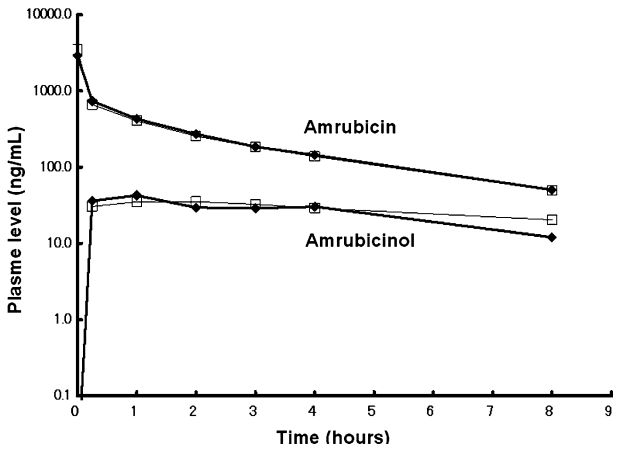

Fig. 1 Plasma disposition curves of amrubicin and its active metabolite, amrubicinol in patients treated at dose level 1 (*filled diamond*) and dose level 2 (*open square*) on day-3, and the results are the mean for three patients (*filled diamond*), and five patients (*open square*), respectively

Pharmacodynamics

Except one patient (point A), a significant relationship was observed between the BI on day-1 and percentage decrease in ANC, using a sigmoid E_{max} model, as illustrated in Fig. 3. The BI_{50} was 0.2303 μg·h/ml, with κ estimated at 1.164. The pharmacodynamic model relating the percentage decrease in ANC to amrubicin AUC did not provide a reasonable fit ($r = 0.10957$; $P = 0.7962$). Amrubicin AUC was hardly related to a decrease in leukocytes ($r = -0.42587$; $P = 0.2928$).

No relationship between the studied parameters and the response or survival was observed.

Response and survival

All eleven patients were assessed for response. Objective responses occurred from the first 25-mg/m^2 dose level of amrubicin combined with a fixed dose of 60 mg/m^2 CPT-11. There were five partial responses (PR), including one good PR in patient with neuroendocrine properties, with an overall response rate of 45%. The median time required to reach remission was 34 days (range: 25–36 days), and the five partial responses ranged in duration between 28 and 87 days (median: 64 days). Four patients showed disease stability, and two patients showed disease progression.

Of the 11 patients, three patients (27%) were still alive as of December 22, 2005. The median progression-free survival was 24 weeks. The median survival time for all 11 patients was 50 weeks.

Discussion

Generally, platinum-based combination chemotherapy is recommended for NSCLC patients with a good performance status [23]. However, the availability of several newer agents with a favorable toxicity profile as

Table 5 Pharmacokinetic parameters of amrubicin, and its active metabolite, amrubicinol determined on days 1 and 3

Amrubicin (mg/m^2)	25		30	
CPT-11 (mg/m^2)	60		60	
	Day 1	Day 3	Day 1	Day 3
Amrubicin	$n = 3$	$n = 3$	$n = 5$	$n = 5$
$T_{1/2Z}$ (h)	2.2 ± 0.13	2.1 ± 0.05	2.3 ± 0.22	2.2 ± 0.19
$AUC_{0\text{-}t}$ (ng h/ml)	1979.3 ± 568.28	2191.0 ± 353.21	1868.2 ± 940.88	1888.8 ± 801.01
$AUC_{0\text{-inf.}}$(ng h/ml)	2116.7 ± 630.15	2331.3 ± 366.02	2005.8 ± 989.68	2020.2 ± 852.40
$AUC_{0\text{-inf.}}/D$ (ng h/ml/mg)	84.7 ± 25.24	93.2 ± 14.65	66.9 ± 32.97	67.5 ± 28.84
$MRT_{0\text{-inf.}}$ (h)	2.4 ± 0.22	2.4 ± 0.18	2.6 ± 0.38	2.5 ± 0.30
Cl_{tot} (l/h/m^2)	12.6 ± 4.24	10.9 ± 1.71	17.1 ± 5.43	16.5 ± 4.86
$V_{d,ss}$ (l/m^2)	30.0 ± 7.08	26.3 ± 6.16	44.5 ± 17.08	41.6 ± 15.39
Amrubicinol	$n = 2$	$n = 3$	$n = 2$	$n = 5$
C_{max} (ng/ml)	34.2 ± 2.12	44.2 ± 10.31	35.1 ± 17.12	40.3 ± 21.08
T_{max} (h)	1.5 ± 0.71	0.8 ± 0.43	2.0 ± 1.00	0.7 ± 0.41
$T_{1/2Z}$ (h)	10.1 ± 1.03	8.3 ± 1.25	7.8 ± 0.53	23.2 ± 18.26
$AUC_{0\text{-t}}$ (ng h/ml)	147.5 ± 86.20	214.8 ± 95.19	148.7 ± 178.03	334.9 ± 410.80
$AUC_{0\text{-inf.}}$(ng h/ml)	513.6 ± 45.61	503.8 ± 133.09	456.0 ± 260.21	1093.1 ± 612.56
$AUC_{0\text{-inf.}}/D$ (ng h/ml/mg)	20.6 ± 1.77	20.1 ± 5.29	15.2 ± 8.70	36.4 ± 20.43

C_{max}: Maximum concentration, T_{max}: Time to reach C_{max}, $T_{1/2Z}$: Half-life in terminal phase, $AUC_{0\text{-}t}$: Area under the plasma concentration-time curve up to last measurable point, $AUC_{0\text{-inf.}}$: Area under the plasma concentration-time curve extrapolated to infinity, $AUC_{0\text{-inf.}}/D$: $AUC_{0\text{-inf.}}$ normalized with dose, $MRT_{0\text{-inf.}}$: Mean residence time extrapolated to infinity, CL_{tot}: Total body clearance, $V_{d,ss}$: Volume of distribution at steady state

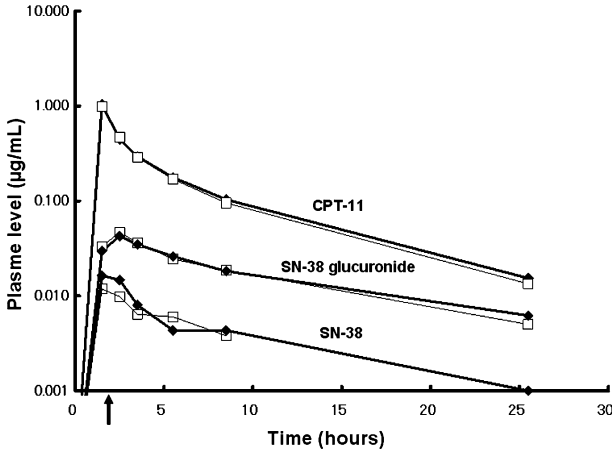

Fig. 2 Pharmacokinetic profile of CPT-11, SN-38 and SN-38G in patients receiving CPT-11 treatment at dose level 1 (*filled diamond*), dose level 2 (*open square*) on day-1, and the results are the mean for three patients (*filled diamond*), and five patients (*open square*), respectively. The *arrow* indicates the completion of infusion

well as innovative, non-overlapping mechanisms of action, makes it possible to explore new treatment programs of non-platinum combination in patients with NSCLC [24]. Although the combined inhibition of topoisomerases I and II is a highly promising scenario, preclinical and clinical trials raised more questions than they answered. In a combination of camptothecins and topoisomerase II inhibitors, the scheduling seems to be critical for success since the sequential administration of camptothecins followed by topoisomerase II inhibitors led to synergistic cytotoxicity [1, 11], while concurrent administration led to antagonism [3, 10, 22]. Therefore, the optimal dosing schedule for these agents remains to be elucidated. The development of a new topoisomerase II inhibitor, amrubicin, has led to renewed interest in a combination of topoisomerase I and II inhibitors. In light of this information, we conducted this Phase I study of CPT-11 and amrubicin for the treatment of previously untreated patients with advanced NSCLC.

This trial demonstrated that the combination of CPT-11 plus amrubicin is feasible and can be given every 21 days in previously untreated patients with advanced NSCLC. The MTD of amrubicin on days 1–3 in combination with 60 mg/m^2 of CPT-11 on days 1 and 8 was 25 mg/m^2, and one of six patients experienced DLT at this dose level (Table 3). The dose of amrubicin was 56% of that reported for a single agent administration as an intravenous injection on days 1–3. The dose of CPT-11 with this schedule was also 60 % of the recommended single-agent dose as a weekly administration, showing that the combined use of the full doses of both agents was not feasible because of overlapping toxicities. Since leukopenia was overlapping DLT in

Table 6 Pharmacokinetic parameters of CPT-11, SN-38 and SN-38 glucuronide determined on days 1 and 8

Amrubicin (mg/m^2)	25		30	
CPT-11 (mg/m^2)	60		60	
	Day 1	Day 8	Day 1	day 8
CPT-11	$n = 3$	$n = 3$	$n = 5$	$n = 3$
$T_{1/2Z}$ (h)	5.8 ± 0.27	5.9 ± 0.18	5.5 ± 0.61	5.8 ± 0.71
$AUC_{0\text{-}t}$ (µg h/ml)	3.8 ± 0.66	3.7 ± 0.68	3.6 ± 1.30	2.9 ± 1.57
$AUC_{0\text{-}inf.}$(µg h/ml)	3.9 ± 0.70	3.9 ± 0.72	3.7 ± 1.38	3.0 ± 1.68
$MRT_{0\text{-}inf.}$ (h)	5.2 ± 0.66	5.3 ± 0.48	4.8 ± 0.88	4.9 ± 1.10
Cl_{tot} (l/h/m^2)	15.7 ± 2.89	15.9 ± 3.02	18.3 ± 7.52	24.1 ± 11.61
$V_{d,ss}$ (l/m^2)	79.7 ± 4.57	84.1 ± 8.41	83.4 ± 22.81	110.9 ± 38.44
SN-38	$n = 3$	$n = 3$	$n = 5$	$n = 3$
C_{max} (µg/ml)	0.02 ± 0.014	0.02 ± 0.009	0.01 ± 0.002	0.02 ± 0.010
T_{max} (h)	1.50 ± 0.000	2.17 ± 0.577	1.50 ± 0.000	1.83 ± 0.577
$T_{1/2Z}$ (h)	3.88 ± 3.446	4.44 ± 5.072	4.91 ± 2.254	4.08 ± 2.736
$AUC_{0\text{-}t}$ (µg h/ml)	0.10 ± 0.131	0.10 ± 0.104	0.05 ± 0.021	0.09 ± 0.103
$AUC_{0\text{-}inf.}$(µg h/ml)	0.12 ± 0.145	0.12 ± 0.131	0.09 ± 0.038	0.10 ± 0.108
SN-38G	$n = 3$	$n = 3$	$n = 5$	$n = 3$
C_{max} (µg/ml)	0.05 ± 0.020	0.05 ± 0.021	0.04 ± 0.020	0.04 ± 0.033
T_{max} (h)	2.50 ± 0.000	2.50 ± 0.000	2.50 ± 0.000	2.17 ± 0.577
$T_{1/2Z}$ (h)	7.27 ± 2.424	8.31 ± 4.227	7.64 ± 2.964	6.15 ± 4.869
$AUC_{0\text{-}t}$ (µg h/ml)	0.40 ± 0.236	0.46 ± 0.294	0.42 ± 0.269	0.37 ± 0.478
$AUC_{0\text{-}inf.}$(µg h/ml)	0.49 ± 0.256	0.59 ± 0.392	0.51 ± 0.352	0.49 ± 0.630

C_{max}: Maximum concentration, T_{max}: Time to reach C_{max}, $T_{1/2Z}$: Half-life in terminal phase, $AUC_{0\text{-}t}$: Area under the plasma concentration-time curve up to last measurable point, $AUC_{0\text{-}inf.}$: Area under the plasma concentration-time curve extrapolated to infinity, $MRT_{0\text{-}inf.}$: Mean residence time extrapolated to infinity, CL_{tot}: Total body clearance, $V_{d,ss}$: Volume of distribution at steady state

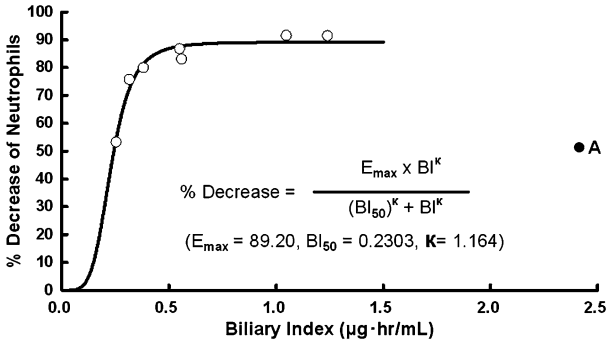

Fig. 3 Pharmacokinetic modeling of percent change in ANC *versus* the biliary index (AUC_{CPT-11} X AUC_{SN-38}/AUC_{SN-38G}) using a sigmoidal maximum-effect (E_{max}) model

both agents, doses was supposed to be reduced from single-agent MTD levels. In the tolerable-dose diagram reported by Korn and Simon [12], this MTD point was just on the leukopenia constraint line for amrubicin and CPT-11, demonstrating that this toxicity was only additive when used in combination. Anyway, the toxic effects were predictable, reversible and manageable. The spectrum of toxicities for amrubicin combined with CPT-11 was similar to that of each agent alone [16, 31]. As expected, diarrhea and missed CPT-11 treatment on day-8 because of leukopenia were the principal DLT of this combination regimen (Table 3). No unexpected toxicity was observed during this trial (Tables 3 , 4). Diarrhea, which could be attributed to CPT-11 [16], was one of DLT in this trial, with four patients having Grade 2 or worse diarrhea. Although leukopenia and neutropenia are typical overlapping toxicities of both drugs [16, 31], no Grade 4 neutropenia lasting more than 3 days occurred in 11 patients. Neither was neutropenia associated with fever noted in these patients. Since neutropenia is the major DLT common to both of these agents, the use of recombinant human granulocyte colony-stimulating factor may be one way to allow higher doses of both agents to be given in combination without incurring significant neutropenia for more chemotherapy-sensitive malignancies including small cell lung cancer. The incidence of cardiotoxicity possibly attributable to amrubicin was null, and has never been a reason for treatment discontinuation. The incorporation of amrubicin instead of doxorubicin in anthracycline-based regimens will potentially reduce the incidence of cardiotoxicity, thereby improving the therapeutic index of doxorubicin-based regimens in future trials.

Since hepatic metabolism and biliary excretion play a principal role in the metabolism and clearance of both amrubicin and CPT-11, a pharmacokinetic drug-drug interaction was theoretically possible. However, the pharmacokinetic results of CPT-11, SN-38, and SN-38G obtained with the present study are in agreement with the results of previously published phase I studies of single agent CPT-11 [6, 13, 15], suggesting that no significant interaction between CPT-11 and amrubicin seemed to occur. Although the concentrations of amrubicinol were frequently below the limit of detection in this assay system, the pharmacokinetics of amrubicin and its active metabolite, amrubicinol, either on day-1 or on day-3 were not modified by the coadministration of CPT-11. Our work to improve the detectable levels of amrubicinol to perform pharmacokinetic study more accurately is now underway.

The response rate of 45% and the median survival time of 50 weeks obtained in this study compared favorably with those previously reported for trials of other combination chemotherapy regimens in patients with NSCLC [24]. Furthermore, this regimen could be an alternative treatment for patients who cannot undergo cisplatin-containing regimens. Since the number of patients in this study is too small to draw any valid conclusion about the ultimate clinical activity of this combination regimen, Phase II trials are needed to allow a precise estimate of the degree of activity of this regimen against advanced NSCLC.

In conclusion, this study showed that amrubicin can be administered at 56% of the recommended single-agent dose in combination with CPT-11 (60 mg/m^2). The major DLT were diarrhea, and leukopenia resulting in CPT-11 dose omission on day-8. In this Phase I study of 11 patients with advanced NSCLC, we observed five partial responses for an encouraging overall response rate of 45%. For future Phase II trials, 25 mg/m^2 of amrubicin (days 1–3) plus 60 mg/m^2 of CPT-11 (days 1 and 8) repeated at 3-week intervals is the appropriate dose and schedule for patients with advanced NSCLC. Furthermore, the results of this study are relevant for diseases other than NSCLC against which CPT-11 and amrubicin show antitumor activity.

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