Cancer Chemother Pharmacol (2006) 58: 173–182
DOI 10.1007/s00280-005-0149-6

ORIGINAL ARTICLE

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Phase I and pharmacokinetic study of edotecarin, a novel topoisomerase I inhibitor, administered once every 3 weeks in patients with solid tumors

Received: 7 June 2005 / Accepted: 29 October 2005 / Published online: 25 November 2005

Abstract *Purpose*: Edotecarin (J-107088) is a potent indolocarbazole topoisomerase I inhibitor which is structurally distinct from the camptothecins. This study aimed to determine the maximum tolerated dose (MTD), the recommended dose for future Phase II studies and the safety, pharmacokinetic profile, and preliminary antitumor activity of edotecarin in a population of patients with advanced solid tumors. *Experimental design*: Edotecarin was administered as a single dose by IV infusion over 2 h every 21 days (with 1 week permitted for recovery from toxicities, if needed) in patients with advanced solid tumors. Doses ranged from 8 to 15 mg/m^2. Pharmacokinetic assessments were performed during and after the first administration. *Results*: Twenty-four patients received 61 cycles of therapy. Dose-limiting toxicities (infection, febrile neutropenia, constipation, ileus, and prolonged grade 4 granulocytopenia) were observed in 3 of 5 evaluable patients at the 15 mg/m^2 dose, defining the MTD. The most commonly reported non-hematologic toxicities were anorexia, nausea, malaise, and constipation. Diarrhea was neither frequent nor severe. Neutropenia was the most common hematologic toxicity (grade 3–4 in 21/23 patients during cycle 1). Plasma concentrations of edotecarin rose rapidly following the start of the 2-hour infusion, reaching C_{max} values of 103 ± 17 ng/ml at the 13 mg/m^2 dose, and decreased steeply after the end of the infusion. Plasma concentrations declined to approximately 1–2 ng/ml at 26 h post start of infusion, the last PK sampling time point. The mean apparent plasma half-life of the drug was 20 h, which should be considered a preliminary estimate until results from studies with a longer duration of plasma sampling are available. A mean of 1.4–3.6% of the dose was recovered as unchanged drug in the urine over 48 h. Unconfirmed tumor regression ≥50% was observed in 2 patients, 1 with metastatic gastric carcinoma and 1 with esophageal cancer. *Conclusions*: The MTD of edotecarin administered IV over 2 h every 21 days was 15 mg/m^2. The recommended dose for Phase II studies with a 3-week schedule (with 1 week permitted for recovery from toxicities, if needed) is 13 mg/m^2. The observed safety profile and preliminary evidence of antitumor activity warrant further investigation of this drug in solid tumors.

Keywords Edotecarin · Pharmacokinetics · Maximum tolerated dose · Dose-limiting toxicity · Topoisomerase I inhibitor

Presented at the American Society of Clinical Oncology 2002 Annual Meeting (abstract no. 385).

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Introduction

DNA damage mediated by topoisomerase I (topo I) inhibitors is an important mechanism of antineoplastic activity [1]. During DNA replication, topo I relieves torsional strain by causing a reversible single-strand

break in the DNA [2]. Topo I binds to DNA at the break site to form a cleavable complex. Topo I inhibitory drugs bind to the topo I-DNA complex, stabilizing it and preventing the religation of the single-strand breaks. Cell death appears to be due to double-strand DNA damage that occurs during DNA synthesis when replication enzymes interact with the stabilized cleavable complex. Currently in the USA, two topo I inhibitors, both camptothecin derivatives, are available for the treatment of cancer patients: topotecan, approved for ovarian carcinoma and small cell lung cancer indications, and irinotecan, approved for metastatic colorectal carcinoma [3–6]. The remarkable anticancer activity of the campothecins and the possibility that other topo I inhibitors could exhibit different activity, better tolerability, or a more favorable pharmacokinetic profile have led to the search for new topo I inhibitors.

Edotecarin (J-107088) is a new derivative of NB-506, an indolocarbazole antitumor agent with a chemical structure completely different from the camptothecins (Fig. 1) [7]. Like camptothecin and its derivatives, edotecarin inhibits topo I [7, 8], but because of the distinct structure of this agent, its interaction with the target enzyme differs significantly from the camptothecin derivatives [9]. Edotecarin is a more potent inhibitor of topo I than camptothecin. The cleavable complex formed with edotecarin is more stable than that with camptothecin and persists significantly longer after removal of drug from cell culture medium [7]. The activity of edotecarin does not appear to be cell-cycle dependent. In vitro edotecarin has demonstrated a wide spectrum of activity against human cancer cell lines [7] and is active in vivo against a variety of human and murine tumor-derived xenografts as well as experimental liver metastases [10, 11]. Preclinical studies in animal species have shown that edotecarin is largely eliminated as unchanged parent drug via biliary excretion (unpublished Banyu data), in marked contrast to the camptothecin analog, irinotecan, which is characterized by a very complex disposition and metabolic pathways. In vitro studies showed that edotecarin was not metabolized by liver microsomes or by hepatocytes from humans or several animal species (unpublished Banyu data). The unique in vitro and in vivo pharmacological profile of edotecarin relative to other topo I inhibitors makes this compound a potentially useful antineoplastic agent.

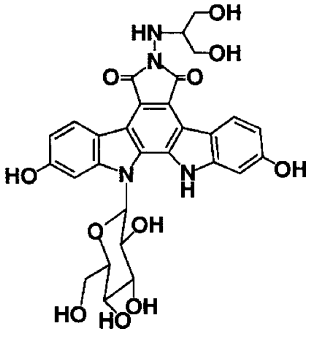

Fig. 1 Structure of edotecarin, an indolocarbazole. Molecular weight: 608.56 Da

When this study was initiated in Japan, edotecarin had been previously investigated in 2 Phase I studies in the USA. In the first [12], edotecarin was administered as a 2 h IV infusion once every 21 days (with an additional week permitted for recovery to ≥grade 1 toxicity, if necessary) at doses of 6, 8, 11, 13 and 15 mg/m^2. Nausea, vomiting, headache, fatigue, febrile neutropenia and neutropenia were dose-limiting at 15 mg/m^2. Treatment could be administered repeatedly for 2 or more courses in 24 of 29 patients (83%). One patient with metastatic bladder cancer had a confirmed partial response of long duration and 12 patients showed stabilization of disease. In the second study [13], edotecarin was administered as a 1 h IV infusion twice-weekly (on days 1, 4, 8, and 11) in cycles of at least 28 days at doses of 2, 4, 5.5, and 7.5 mg/m^2/day. Mucositis, neutropenia and thrombocytopenia were dose-limiting at 7.5 mg/m^2/day. Only two patients received treatment beyond cycle 2.

Based on these results, this Phase I study was designed to evaluate ascending doses of edotecarin administered by 2 h IV infusion every 21 days. A more sensitive assay was utilized in this study compared to the earlier study, of similar design, conducted in the USA. Objectives of the study were to determine the MTD (where the MTD is the maximum dose administered) and the dose to be recommended in future Phase II studies, and to assess its safety, pharmacokinetic profile, and the preliminary antitumor activity in a population of Japanese patients with advanced solid tumors.

Patients and methods

Patients

Japanese patients with histologically or cytologically confirmed evaluable malignant solid tumors refractory to conventional chemotherapy or tumors for which no effective therapy existed were candidates for this study. Inclusion criteria also included the following: age ≥20 years; Eastern Cooperative Oncology Group (ECOG) performance status 0, 1, or 2; life expectancy ≥12 weeks; absolute granulocyte count ≥1,500/mm^3, platelet count ≥100,000/mm^3, hemoglobin ≥9 g/dl, and serum creatinine <1.5 mg/dl. Additional entry criteria were serum total bilirubin within the normal limit and serum AST, ALT, and alkaline phosphatase less than twice the upper limit of normal. A 4-week interval was required for chemotherapy, radiation therapy, or immunotherapy treatments (6-week interval for patients previously treated with mitomycin C or nitrosoureas). A 2-week interval after major surgery was required.

Patients had to have fully recovered from toxicity associated with previous therapy. Patients were ineligible for the study if they had symptomatic central nervous system metastases, neurological symptoms, fever, or unstable significant clinical conditions. Patients who were receiving corticosteroids, anticoagulants, immunotherapy, biological response modifiers, or other investigational agents; or who had received prior radiation therapy to 20% greater of bone marrow; or who had received a bone marrow stem cell transplant, were excluded from the study.

The protocol was approved by the institutional review boards of the National Cancer Center Hospital and the Nagoya Medical Center, and all patients gave written informed consent prior to study entry.

Dosage and dose escalation

Edotecarin (J-107088) was provided as an injectable preparation in plastic infusion bags by Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan). Each bag contained 37.5 mg edotecarin in a 250 ml 5% glucose solution (final edotecarin concentration of 0.15 mg/ml). Edotecarin was administered by intravenous (IV) infusion over 2 h every 21 days. Patients were hospitalized for the initial course of edotecarin and remained hospitalized for close observation for 21–28 days thereafter. Subsequent courses could be administered on an outpatient basis with a weekly evaluation by the investigator.

The initial dose of edotecarin was 8 mg/m^2, and subsequent doses were escalated in approximately 33% increments (to 11 and 15 mg/m^2). Patients were enrolled in cohorts of 3 patients per dose and observed for 21 days; the observation period was extended to 28 days if a longer recovery period was needed. If only one of the three patients experienced a dose-limiting toxicity (DLT), then three additional patients were treated at the same dose. If none of the first three patients in a cohort, or if only one of six patients demonstrated DLT, then the next three patients were treated at the next higher dose. If at least two patients in the cohort experienced DLT, that dose level was regarded as the MTD. The dose for the next cohort would then be reduced by approximately 15%. The recommended dose for future Phase II studies was to be evaluated in a total of nine patients and was to be the highest dose at which fewer than a third of treated patients experienced a DLT. Individual patients who did not experience DLT and had no evidence of disease progression could receive up to four courses of edotecarin at the dose originally assigned. No intrapatient dose escalation was permitted. Patients who had a DLT that had recovered to grade 2 or less could continue treatment with edotecarin at a dose below the dose at which DLT was demonstrated. If DLT occurred at the reduced dose, no further treatment was to be administered. Patients with progressive disease were to discontinue treatment.

Definition of dose limiting toxicity (DLT)

For the purpose of this study, DLT was defined as the occurrence of pre-specified severe adverse events [severity defined according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) version 2.0], occurring during cycle 1 and attributed to edotecarin. Criteria that are relevant to DLT's observed in this study were: grade 3 or 4 non-hematologic toxicity (except nausea, vomiting, fever and fatigue effectively managed with symptomatic treatment, and alopecia); grade 4 granulocytopenia accompanied by fever of ≥39.1°C, or accompanied by an infection requiring antibiotic or antifungal treatment based on fever of ≥38.0°C, or that persisted ≥5 days; grade 4 leukopenia that persisted ≥5 days; or failure of granulocyte and platelet counts to return to ≥1,500/mm^3 and ≥100,000/mm^3, respectively, within 28 days after edotecarin administration.

Supportive care

Each patient received granisetron 3 mg and dexamethasone 20 mg IV pretreatment on day 1 and dexamethasone 8 mg IV on days 2–4 post treatment for prevention of nausea, vomiting, and general malaise. Granisetron 3 mg IV was also administered on days 2–4 if needed. Routine use of colony-stimulating factors was not permitted during cycle 1. However, patients who had granulocytopenia that had met the criteria for DLT were permitted to receive filgrastim in cycle 1 and subsequent cycles.

Patient evaluation

Patients were evaluated at baseline and periodically throughout the study. During the first cycle, vital signs were measured every 1–3 days, hematology determinations were performed every 2–3 days, and serum biochemistries on days 3, 8, and 15. Physical examinations, including evaluation of performance status and measurement of palpable tumors, were done on days 8 and 15. During the second and the subsequent cycles, vital signs, laboratory tests and toxicity evaluations were performed on days 1, 8, and 15 of each cycle. For cycle 1, blood coagulation studies were done before each dose and on days 8 and 15. Measurements for subsequent cycles were on day 1, and finally, within 2 weeks after terminating the study. Performance status was assessed by the physician according to the ECOG criteria [14]. Tumor responses were based on WHO criteria. Radiographic evaluations of tumor size were performed every two cycles and were to be repeated after 4 weeks in case of response. Safety events were recorded on the basis of changes in signs and symptoms, physical findings, vital signs, and laboratory abnormalities. Weekly severity

assessments of subjective and objective findings were performed according to the NCI CTC version 2.

Plasma edotecarin measurements and assay

Pharmacokinetic analysis

Pharmacokinetic studies were performed during the first cycle of treatment. On day 1, blood samples (6 ml each) were drawn into heparinized tubes from an indwelling IV cannula in the arm contralateral to the arm bearing the infusion line. Samples were collected before infusion, at 15 and 60 min after the start of the infusion, at the end of the 2-h infusion, and at 5, 15, 30, and 60 min and 2, 4, 6, 8, 10 and 24 h after the end of the infusion. Urine samples were collected over two 24-h intervals for 48 h after the start of the infusion.

The concentrations of edotecarin in plasma and in urine were analyzed by Mitsubishi Chemical BCL (Tokyo) using validated, high-performance liquid chromatography. J-109404, a chemical analog of edotecarin, was the internal standard. BondElut CH cartridges were pre-conditioned with successive 1-ml washes of dichloromethane, methanol, and water. Plasma samples (1 ml) were mixed with 0.1 ml of 10% acetonitrile, 0.1 ml of the internal standard (1 μg/ml) in 10% acetonitrile, and 1 ml of 50 mM, pH 7.0 phosphate buffer and applied to pre-washed BondElut cartridges. The cartridges were then washed with 1 ml of water, spun at 1,000 rpm for 1 min at 4°C, washed again with 1-ml of 20% methanol, and spun again. To each column was then added 0.5 ml of 60% methanol followed by centrifugation to elute the retained compounds of interest. Eluates were dried under a nitrogen stream, reconstituted in 0.1 mL of mobile phase, and transferred to Ultrafree MC (0.2 μm) centrifugal filter units. The filter units were spun at 15,000 rpm for 5 min at 4°C and 60 μl of the filtrate was chromatographed. Chromatography was carried out on a Superiorex ODS S-5 μm, 4.6 mm ID×250 mm column with a Capcell C18 UG120, 4 mm ID×10 mm guard cartridge (source of columns was Shiseido, Tokyo) eluted with water/acetonitrile/methanol/trifluoroacetate (TFA) (67/18/15/0.1) flowing at 1.0 ml/min. The ultraviolet absorbance of the effluent was monitored at 334 nm. Linearity was demonstrated over the range of 1–500 ng/ml. Assays of quality control samples (each assay run included 2 replicate QC samples each at 2.5, 40.0, and 400.0 ng/ml) were within ±20% of nominal concentrations, with one exception: a single 2.5 ng/ml replicate assayed at 1.6 ng/ml in 1 assay run. The lower limit of quantitation (LLOQ) was 1.0 ng/ml. The interday and intraday coefficients of variation for plasma are 2.0∼5.9% and 2.0∼4.0%, respectively.

Extraction and chromatography of urine samples was carried out with slight modifications of the plasma method. The urine volume assayed was 0.1 ml, which was mixed with 0.1 ml of 10% acetronitrile, 0.1 ml of 2 μg/ml internal standard in 10% acetonitrile, and 0.2 ml of mM phosphate buffer, pH 7.0. Extraction was carried out using pre-washed BondElut CN columns. The mobile phase consisted of acetonitrile/water/TFA (75/25/0.1) flowing at 0.8 ml/min. The absorbance of the effluent was monitored at 430 nm. LLOQ was 50 ng/ml. Performance of the urine assay was comparable to that of the plasma assay. The interday and intraday coefficients of variation for urine are 1.1∼8.5 and 3.3∼6.6%, respectively.

Adequate freeze/thaw, frozen storage, and autosampler stability were demonstrated for both plasma and urine samples. Stability for plasma and urine was demonstrated after three cycles of freezing and thawing; at −70°C for 3.5 months; and for 24 h while in the autosampler during the assays.

Statistical analysis

Noncompartmental pharmacokinetic parameters were computed using WinNonlin, version 3.1 software (Pharsight Corporation, Mountain View, CA, USA) [15]. An apparent terminal half-life ($t_{1/2}$) was calculated from plasma concentrations observed at 8, 10, and 24 h after the end of the infusion. Dose proportionality was analyzed by means of a power model: $AUC_{0\text{-}\infty} = A \times dose^B$ and $C_{max} = C \times dose^D$, where A and C are proportionality constants, dose units are milligram/patient, and the 95% confidence limits around B and D include 1 if AUC and C_{max} are directly proportional to dose. Nonlinear regression of $AUC_{0\text{-}\infty}$ and C_{max} versus dose and 95% confidence limits on computed parameters B and D were computed using GraphPad Prism, version 4 (GraphPad Software, San Diego, CA, USA). Descriptive statistics (Table 6) were also computed using GraphPad Prism.

Results

Patient characteristics

Twenty-four Japanese patients were enrolled into the study (Table 1) and received at least one dose of edotecarin. The median age of the patients was 56 years (range 33–72), 22 (92%) had an ECOG performance status of 1 at baseline, and 16 (67%) patients were male. The most common tumor types were lung and colorectal cancer. All patients except one had previously received chemotherapy with a median number of chemotherapy regimens of 2 (range 0–4). Patients treated at various dose levels had similar baseline characteristics.

All 24 patients were assessed for both safety and efficacy.

A total of 61 cycles of treatment were administered in these patients. The majority of cycles were administered every 3 weeks, with 10 cycles administered at day 28. Three cycles were administered beyond this to accommodate patients' personal reasons and schedules. The

Table 1 Patient characteristics

Number enrolled	24
Age (years)	
Median	56
Range	33–72
Male:female (no. of patients)	16:8
ECOG performance status (no. of patients)	
0	2
1	22
Tumor type (no. of patients)	
Lung	7
Colorectal	4
Uterine sarcoma	3
Gastric	2
Bile duct	2
Primary unknown	2
Other	4
Prior treatment	
Chemotherapy	
No. of prior regimens (no. of patients)	
0	1
1	11
2	8
3	3
4	1
Median (no. of regimens)	2
Surgery (no. of patients)	11
Radiation therapy (no. of patients)	5
Chemotherapy + radiation therapy (no. of patients)	5

median number of cycles at the 13 mg/m^2 dose was 2 (range 1–6). The most common reasons for treatment discontinuation were progressive disease or best response achieved (i.e., further treatment effect not expected) (nine patients each) (Table 2).

Dose escalation and identification of DLT, MTD, and the recommended Phase II dose

At the initial dose of 8 mg/m^2, no patient experienced DLT. At the next dose of 11 mg/m^2, 1 of 6 patients experienced DLTs (grade 3: infection, febrile neutropenia, and hypoxia). Six patients were enrolled at 15 mg/m^2 . Three patients experienced DLTs (grade 3: infection, febrile neutropenia, and constipation in 1 patient; grade 3: ileus and constipation in the second; and grade 4 granulocytopenia persisting for 5 days or more in the third). Both patients who had grade 3 constipation had previous intestinal surgery for rectal and colon cancer, respectively. One patient with superior vena caval syndrome at baseline required radiotherapy at day 7 for progression of this syndrome. The dose of 15 mg/m^2 was defined as the MTD.

Consequently, 9 patients were treated at 13 mg/m^2. Two of the nine patients treated at this dose experienced DLTs (grade 4 granulocytopenia persisting for 5 days or more in both patients). Therefore, based on protocol-predefined criteria, 13 mg/m^2 is the dose recommended for future single-agent Phase II studies with an administration every 21 days (with an additional week permitted for recovery from toxicities, if needed).

Non-hematologic toxicity

The non-hematologic adverse events reported most commonly (occurrence in ≥50% of all patients treated) during the first cycle were anorexia, nausea, malaise, and constipation (Table 3). No grade 4 non-hematologic toxicities were reported in any treatment group. During subsequent cycles, adverse events were similar in terms of frequency and severity to those reported during the first cycle of treatment and no cumulative toxicity was observed.

Gastrointestinal toxicity, usually mild, was the most common non-hematologic toxicity associated with edotecarin administered with an antiemetic regimen. The median time to onset of constipation during the first cycle was 3 days (range: 2–4 days). None of the patients who received antiemetic prophylaxis and who were treated at the Phase II recommended dose of 13 mg/m^2 had grade 2 or higher nausea, vomiting, or anorexia. Diarrhea (grade 1–2) was observed in only a few patients (8 mg/m^2, 1 patient; 11 mg/m^2, 2; 13 mg/m^2, 2; and 15 mg/m^2, 1). Alopecia was not reported. Injection site phlebitis (grade 2) was reported in 11 (48%) of the patients. The frequency of this event was not dose-dependent, suggesting that it was related to the infusion

Table 2 Number of treatment cycles, number of patients with dosing interval extensions and reasons for treatment discontinuation

	Edotecarin dose (mg/m^2)			
	8	11	13	15
Number of patients	3	6	9	6
Median number of treatment cycles (range)	3 (1–4)	2 (1–4)	2 (1–6)	1 (1–5)
Dose delay				
Number of patients (number of days in dosing interval)	0	1 (45)	2 (31–34)	0
Reasons for treatment discontinuation				
Progressive disease	1	3	4	1
Further treatment effect not expected	2	2	3	2
DLT	0	1	1	2
Adverse events other than DLT	0	0	1	0
Prohibited concomitant therapy	0	0	0	1

Table 3 Most common non-hematologic toxicities occurred in ≥50% of all patients treated during cycle 1

Adverse event	Grade	Dose (mg/m^2)				All doses ($N=24$)
		8 ($N=3$)	11 ($N=6$)	13 ($N=9$)	15 ($N=6$)	
Anorexia	1/2	3 (100%)	6 (100%)	7 (78%)	4 (67%)	20 (83%)
	3/4	0	0	0	0	0
Nausea	1/2	2 (67%)	2 (33%)	5 (56%)	6 (100%)	15 (63%)
	3/4	0	1 (17%)	0	0	1 (4%)
Malaise	1/2	3 (100%)	4 (66%)	5 (56%)	4 (67%)	16 (67%)
	3/4	0	0	0	0	0
Constipation	1/2	1 (33%)	1 (17%)	6 (67%)	3 (50%)	11 (46%)
	3/4	0	0	0	2 (33%)	2 (8%)

procedure rather than the drug. There were no deaths within 28 days of edotecarin administration, and none of the deaths that occurred after the study were considered treatment-related.

One adverse event deserves a more detailed description. A 44 year-old-male with esophageal cancer with liver and lung metastases developed grade 2 interstitial pneumonitis 2 weeks after administration of edotecarin 13 mg/m^2. He had undergone radiation therapy for the primary lesion approximately 4 months before edotecarin infusion. Most lesions of the interstitial pulmonary lesions were in the field of radiation therapy, and radiation pneumonitis was diagnosed. However, the rapid onset of pneumonitis after edotecarin infusion suggested a recall phenomenon induced by edotecarin and the event was judged to be possibly related to the study treatment.

Hematologic toxicity

Neutropenia/granulocytopenia, leukopenia, anemia, and lymphocytopenia were the most common (occurrence in ≥50% of all patients treated at the 3 highest dose levels) hematologic toxicities reported during the first cycle (Table 4). Neutropenia was the principal hematologic toxicity in this study and granulocytopenia was dose-limiting at 13 and 15 mg/m^2 (Table 5). At 11–15 mg/m^2, the median time to nadir granulocyte count was 11–14 days, and the median time to recovery from nadir was 5 to 7 days. In all patients, granulocyte counts recovered to grade 1 within 21 days after edotecarin infusion at 8 and 15 mg/m^2, but had not recovered by day 22 in 1 patient at 11 mg/m^2 and in 4 patients at 13 mg/m^2. However, by day 26 after edotecarin infusion, granulocyte counts were within normal limits in all patients. Given this frequent and severe neutropenia, G-CSF support was provided during cycle 1 in 2 patients at 13 mg/m^2 and 1 at 15 mg/m^2,after DLT confirmation. Grade 4 granulocytopenia persisting for more than 5 days led to dose reduction in the second cycle in 2 patients, one each at 13 and 15 mg/m^2.

Grade 4 neutropenia (not necessarily lasting > 5 days), was reported in 15 (65%) patients during the first cycle of therapy, and was observed at all dose levels but did not increase in incidence with additional cycles.

Anemia, reported in 15 (65%) patients, did not exceed grade 1 or 2 in severity. Thrombocytopenia was not reported in this study. The numbers of patients with abnormal laboratory values did not tend to increase with increasing courses of treatment, suggesting that the toxicity of edotecarin was not cumulative.

No significant changes were demonstrated by the blood coagulation studies, which assessed prothrombin time (PT) and activated partial thromboplastin time (aPTT).

Pharmacokinetics

Plasma pharmacokinetic parameters are listed in Table 6. Edotecarin plasma concentrations rose rapidly

Table 4 Most common hematologic toxicities occurred in ≥50% of patients at each of the three highest dose levels during cycle 1

Adverse event	Grade	Dose, mg/m^2				All doses ($N=23$)
		8 ($N=3$)	11 ($N=6$)	13 ($N=9$)	15 ($N=5$)	
Neutropenia	1/2	1 (33%)	0	0	0	1 (4%)
	3/4	1 (33%)	6 (100%)	9 (100%)	6 (100%)	22 (92%)
Granulocytopenia	1/2	1 (33%)	0	0	1 (17%)	2 (8%)
	3/4	1 (33%)	6 (100%)	9 (100%)	5 (83%)	21 (88%)
Leukopenia	1/2	1 (33%)	4 (67%)	5 (56%)	2 (33%)	12 (50%)
	3/4	0	2 (33%)	4 (44%)	4 (67%)	10 (42%)
Anemia	1/2	3 (100%)	4 (67%)	5 (56%)	4 (67%)	16 (67%)
	3/4	0	0	0	0	0
Lymphocytopenia	1/2	1 (33%)	4 (67%)	4 (44%)	2 (33%)	11 (46%)
	3/4	0	0	1 (11%)	2 (33%)	3 (13%)

Table 5 Granulocytopenia during cycle 1

Dose (mg/m^2)	No. of patients	Grade				Nadir ($no./mm^3$)[a]	Days to nadir[a]	Days to recovery (grade 0) from nadir			
								Without G-CSF		With G-CSF	
		1	2	3	4			No. of patients	No. of days[a]	No. of patients	No. of days[a]
8	3	0	0	1 (33%)	0	2,193 (550–993)	18 (14–21)	1	7	0	–
11	6	0	0	2 (33%)	4 (67%)	440 (150–923)	14 (11–16)	6	7 (3–12)	0	–
13	9	0	0	4 (44%)	5 (56%)	480 (46–912)	14 (9–21)	7	7 (2–13)	2	5 (4–5)
15	6	0	1 (17%)	2 (33%)	3 (50%)	529 (88–1,017)	11 (11–14)	4	5 (2–7)	1	5

G-CSF granulocyte colony-stimulating factor
[a]Expressed as median (range)

Table 6 Pharmacokinetic parameters in cycle 1 (mean ± SD)

Dose (mg/m^2)	No. of patients[c]	C_{max}, (ng/ml)	$AUC_{0\text{-}26h}$, (ng h/ml)	$AUC_{0\text{-}\infty}$ (ng h/ml)	Percentage of area extrapolated	CL		$t_{1/2}$ (h)	V_{SS} (l/m^2)	48-h urinary recovery (%)
						$ml/min/m^2$	ml/min			
8	2	57 ± 17[a]	132, 192	330, 198	42, 23	404, 673	735, 1043	45.5, 29.0	972, 715	1.4 ± 2.3[a]
11	5	86 ± 10[b]	211 ± 38	294 ± 54	25 ± 8	624 ± 114	962 ± 287	26.1 ± 3.3	670 ± 277	3.6 ± 2.7
13	9	103 ± 17	262 ± 43	330 ± 44	19 ± 6	657 ± 88	1154 ± 138	22.9 ± 2.1	569 ± 256	2.9 ± 3.8
15	5	113 ± 17[b]	269 ± 56	352 ± 62	20 ± 5	711 ± 123	1057 ± 190	20.2 ± 1.6	561 ± 258	1.8 ± 3.4[b]

C_{max}, maximum plasma concentration; *AUC* area under the plasma concentration-time curve; *CL* clearance; $t_{1/2}$, apparent terminal half-life; V_{ss}, steady-state volume of distribution
[a]$N = 3$
[b]$N = 6$
[c]As drug concentrations were below the detectable range at one or more time points beyond the 8 h after the administration, some parameters are not available for all patients

at the start of the infusion and dropped sharply at discontinuation of the infusion (Fig. 2), reaching levels of 1–2 ng/ml at the last sampling point.

In order to compare PK data from this study, to PK data from the similar US study, a noncompartmental model for calculating PK parameters was used.

Peak plasma concentrations of edotecarin and $AUC_{0\text{-}26h}$ increased with increasing dose (Fig. 3). Power model fitting to $AUC_{0\text{-}\infty}$ and C_{max} vs dose data suggested that C_{max} was proportional to dose while $AUC_{0\text{-}\infty}$ was somewhat less than dose-proportional (results not presented).

Clearance (CL) values were comparable at 11, 13 and 15 mg/m^2 as were volumes of distribution.

An apparent terminal half-life ($t_{1/2}$) was calculated from plasma concentrations observed at 8, 10, and 24 h after the end of the infusion. In three patients, the plasma concentration at 1 or more of these time points was below the lower limit of quantification of 1 ng/ml; consequently, values of $t_{1/2}$, $AUC_{0\text{-}\infty}$, and CL were not calculated in these patients. The mean apparent $t_{1/2}$ was approximately 20 h. Since the PK sampling duration was only 26 h post start of infusion (i.e., for only about 1 half-life), the computed $AUC_{0\text{-}\infty}$, CL, and $t_{1/2}$ values should be considered preliminary until data are available from trials measuring plasma levels through at least 3–4 times the apparent terminal $t_{1/2}$.

Mean 48-h urinary excretion accounted for 1.4–3.6% of the administered dose of edotecarin in the 4 cohorts.

Coefficient of interpatient variation in edotecarin C_{max} and AUC values was 15–20%.

Antitumor activity

All 24 patients were assessable for efficacy, and 21 patients had evaluable lesions. No objective and confirmed responses were observed. However, stable disease (SD) was reported in 12 patients (57%), with a maximum duration of 5 cycles. Eight (38%) patients had tumor volume reductions ranging from 10.5 to 65.4% based on the sum of the products of the dimensions of measurable lesions. Two patients had a decrease in tumor volume of at least 50%. One patient, enrolled at 13 mg/m^2, had esophageal cancer with liver metastases. After cycle 1, at day 18, tumor volume was reduced by 65%. Unfortunately, treatment was discontinued because of pneumonitis and the disease had progressed 28 days later. The second patient, who received 11 mg/m^2, had gastric cancer with measurable metastatic lesions in the lung

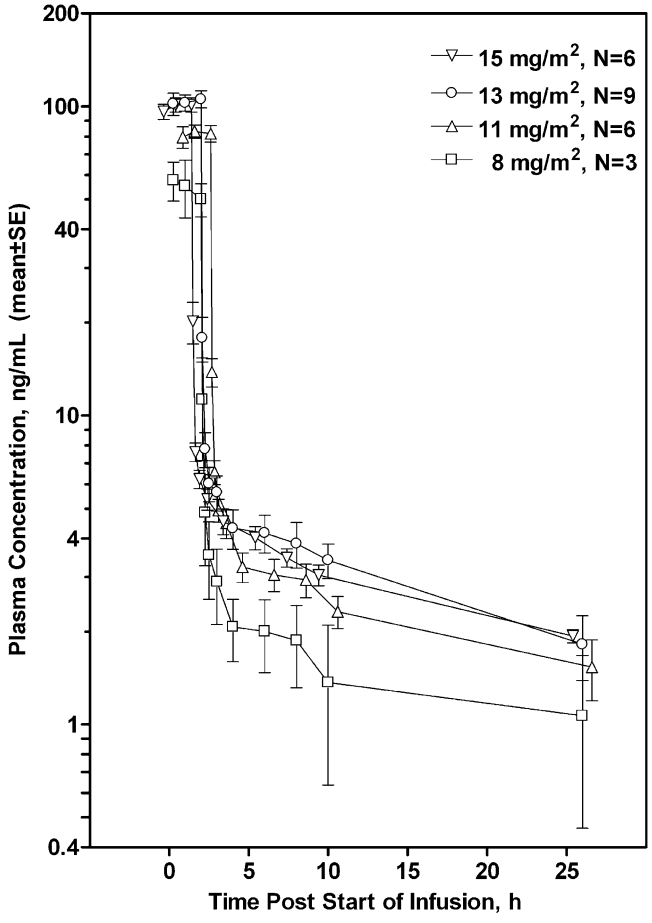

Fig. 2 Edotecarin cycle-1 plasma concentration-time profiles (mean ± SE) in patients with advanced solid tumors. Data at 11 and 13 mg/m^2 have been offset by +0.6 and −0.6 h, respectively, in the *x*-direction to prevent overlapping *data points* and *error bars*

and mediastinal lymph nodes. These lesions decreased gradually in size, with the reduction from baseline reaching 53% by cycle 3. For administrative reasons, cycle 4 was delayed (45 days). The response was no longer present and the treatment was discontinued. Three of four patients with colorectal cancer, all with lung lesions and having received prior 5-fluorouracil treatment, showed evidence of tumor stabilization.

Discussion

The first objective of this Phase I clinical study was to determine the MTD of single-agent edotecarin administered by IV infusion over 2 h once every 21 days with pre- and post-treatment supportive medication (steroids and 5-HT$_3$ antagonists). The MTD was 15 mg/m^2 with this schedule of administration. At this dose, hematologic (neutropenia, febrile neutropenia) and non-hematologic (constipation, ileus, infection without neutropenia) grade 3 and 4 toxicities were observed in 3 of the 6 patients of the MTD cohort.

In order to make a recommendation regarding the dose of edotecarin to be studied in future single-agent Phase II studies, 9 patients were treated at 13 mg/m^2 with supportive medication. Grade 4 granulocytopenia, lasting for 5 days or more, was observed in only 2 patients. Therefore this dose is recommended, with supportive medication, for future Phase II studies. A similar Phase I edotecarin trial conducted in the USA recommended the same dose [12].

At the recommended dose of 13 mg/m^2, edotecarin, infused IV for 2 h with supportive medication, had manageable toxicities. Neutropenia was dose-dependent

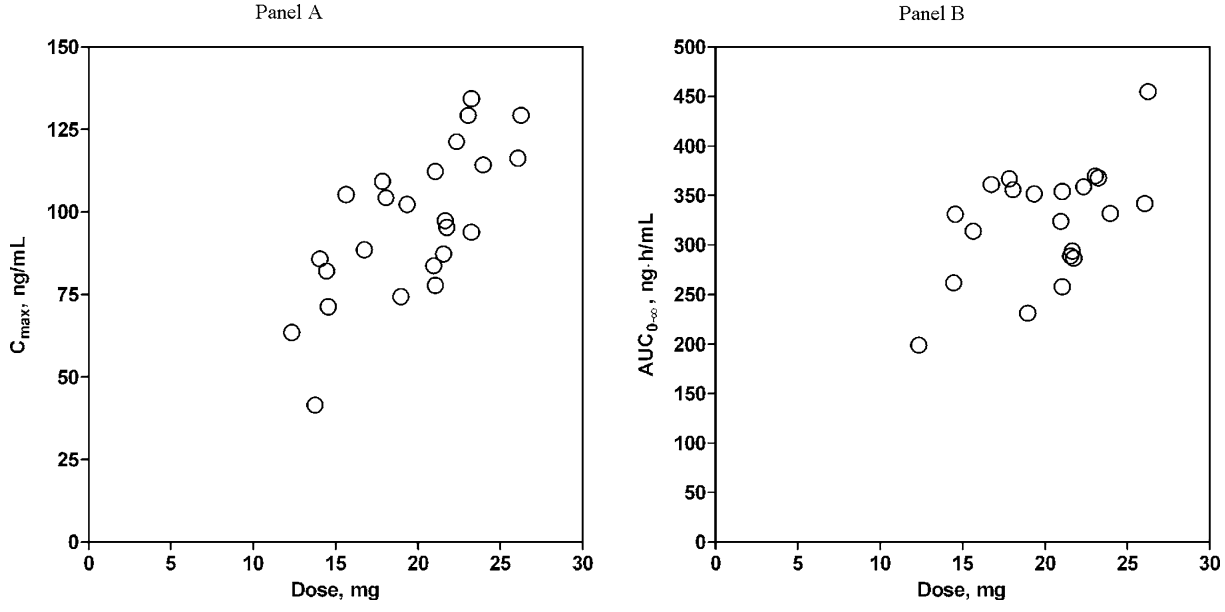

Fig. 3 Peak plasma concentrations (*left panel*) and $AUC_{0-\infty}$ values (*right panel*) as a function of dose in milligram in patients with advanced solid tumors

but was not cycle-dependent. At the recommended dose, the median time to neutrophil nadir was 14 days with a median recovery time of 7 days. This suggests that administration of edotecarin every 3 weeks is possible, but bone marrow recovery has to be verified before the administration of the next cycle. Other hematologic toxicities were observed (anemia, lymphocytopenia), but thrombocytopenia was not reported.

Gastrointestinal toxicity, the most common non-hematologic toxicity observed, was usually mild. At the recommended dose and with antiemetic premedication, nausea, vomiting or anorexia never exceeded grade 1. The incidence of constipation was dose dependent and dose-limiting at 15 mg/m^2 in 2 patients that had previously undergone intestinal surgery for cancer. However, at the recommended dose, constipation was never more severe than grade 2. In two previous Phase I trials of edotecarin [12, 13], constipation was not dose-limiting. The reason for these discrepant observations is unclear, but constipation is not infrequent in colorectal cancer patients receiving chemotherapy. For instance, the incidence of constipation in colorectal patients treated with irinotecan single agent in second line therapy is estimated between 8 and 10% [16]. Because of the relatively high frequency and the dose dependency observed in this study, frequency of bowel movements should be monitored in patients enrolled in future studies with edotecarin. Alopecia and severe diarrhea frequently observed in association with other chemotherapy drugs (e.g., camptothecins) were not seen in this trial.

At the recommended dose, 1 patient, who had received thoracic radiotherapy for lung metastasis of an esophageal carcinoma, had a grade 2 interstitial pneumonitis shortly after edotecarin infusion, suggesting a recall phenomenon. Interstitial pneumonitis has also been observed after irinotecan [17, 18]. Pulmonary toxicity should be closely monitored in patients previously treated with thoracic radiation therapy in future Phase II trials of edotecarin.

Conclusions, based on the pharmacokinetic data, are tentative because of the narrow dose range and small number of patients studied in this trial, as well as the relatively short duration of PK sampling. While plasma concentrations appeared to reach a plateau during the 2-h infusion, attainment of a steady state concentration (C_{ss}) would not be expected for a drug with the multi-compartmental disposition behavior exhibited in Fig. 2. In theory, an infusion duration of 3–4 times the apparent terminal $t_{1/2}$ of 20 h would be required to achieve C_{ss}. This is supported by the fact that an attempt to compute CL by the equation $CL = k_0/C_{max}$, where k_0 is infusion rate and C_{max} is used as an estimate of C_{ss}, yielded CL values much higher than those calculated by means of $CL = dose/AUC_{0-\infty}$ (results not presented).

Within the limited dose range of 11–15 mg/m^2, C_{max} and AUC appeared to increase roughly linearly with dose, and CL was not relatively changed. These results suggest that edotecarin exhibits linear pharmacokinetics within 11–15 mg/m^2.

Urinary recovery did not appear to vary with the dose, which indicates that renal elimination contributes minimally to total body CL.

The pharmacokinetic profile of edotecarin appears to be simple with relatively little interpatient variability compared to that of irinotecan (a prodrug topo-I inhibitor with a very complex disposition) and to many other cytotoxic drugs.

Efficacy was a secondary endpoint of this trial conducted in a population of heavily pretreated cancer patients. No partial or complete confirmed responses were observed. However 8 patients had minor reductions in tumor size, including 2 patients with $>50\%$ regression of tumor size, however these reductions were not confirmed. Twelve patients qualified as having SD. Further clinical work is necessary to quantify precisely the level of edotecarin antitumor activity.

In conclusion, this study showed that the MTD of edotecarin, administered as a 2 h infusion with supportive medication of steroids and antiemetics, is 15 mg/m^2 and the dose to be studied in future Phase II trials as a single agent with an administration every 21 days is 13 mg/m^2. At 13 mg/m^2, toxicities, mainly neutropenia, are manageable and are characterized by the lack of severe diarrhea. The pharmacokinetic profile is attractive. Edotecarin is a promising new anticancer agent, especially in colorectal cancer, and deserves further clinical evaluation.

Acknowledgements We acknowledge Ms. Sayaka Miyahara, Dr. Mami Matsumoto, Ms. Yoko Kawamura, Mr. Katsuhiro Mizuno, and Mr. Naoyoshi Yatsuzuka for pharmacokinetic analyses, data management, and clinical research coordination, and Drs. Claude George and Samit Hirawat for their contribution in preparation of this manuscript.

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