Phase I and Pharmacokinetic Study of DE-310 in Patients with Advanced Solid Tumors

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ABSTRACT

Purpose: **To assess the maximum-tolerated dose, toxicity, and pharmacokinetics of DE-310, a macromolecular prodrug of the topoisomerase I inhibitor exatecan (DX-8951f), in patients with advanced solid tumors.**

Experimental Design: **Patients received DE-310 as a 3-hour infusion once every 2 weeks (dose, 1.0-2.0 mg/m^2) or once every 6 weeks (dose, 6.0-9.0 mg/m^2). Because pharmacokinetics revealed a drug terminal half-life exceeding the 2 weeks administration interval, the protocol was amended to a 6-week interval between administrations also based on available information from a parallel trial using an every 4 weeks schedule. Conjugated DX-8951 (the carrier-linked molecule), and the metabolites DX-8951 and glycyl-DX-8951 were assayed in various matrices up to 35 days post first and second dose.**

Results: **Twenty-seven patients were enrolled into the study and received a total of 86 administrations. Neutropenia and grade 3 thrombocytopenia, and grade 3 hepatotoxicity with veno-occlusive disease, were dose-limiting toxicities. Other hematologic and nonhematologic toxicities were mild to moderate and reversible. The apparent half-life of conjugated DX-8951, glycyl-DX-8951, and DX-8951 was 13 days. The area under the curve ratio for conjugated DX-8951 to DX-8951 was 600. No drug concentration was detectable in erythrocytes, skin, and saliva, although low levels of glycyl-DX-8951 and DX-8951 were detectable in tumor biopsies. One patient with metastatic adenocarcinoma of unknown primary achieved a histologically proven complete remission. One confirmed partial remission was observed in a patient with metastatic pancreatic cancer and disease stabilization was noted in 14 additional patients.**

Conclusions: **The recommended phase II dose of DE-310 is 7.5 mg/m^2 given once every 6 weeks. The active moiety DX-8951 is released slowly from DE-310 and over an extended period, achieving the desired prolonged exposure to this topoisomerase I inhibitor.**

INTRODUCTION

DE-310 is a novel macromolecular drug delivery system for the topoisomerase I inhibitor DX-8951 (exatecan mesylate), in which each molecule is linked via a glycyl-glycyl-phenylalanyl-glycyl-peptidyl spacer to a biodegradable carboxymethyldextran polyalcohol polymer. This carrier part of DE-310 is intended to provide passive accumulation in tumor tissue and sustained release of the active moiety DX-8951 within the tumor, which occurs as result of enzymatic cleavage of the peptidyl spacer by cathepsin B and cathepsin L, thereby enhancing its activity and reducing its systemic toxicity (1). The main chain is acid labile, suggesting that it is biodegradable and can be depolymerized after endocytosis in a lysosomal acidic-environment (Fig. 1; ref. 1).

The cytotoxicity of topoisomerase I inhibitors in animal models increases with duration of exposure, and long-term exposures to low concentrations are more effective than short-term exposures to high concentrations (2). The concept behind the development of DE-310 is that the macromolecular carrier will accumulate and be retained preferentially in tumor tissue by an enhanced permeability and retention effect (3–5).

Preclinical studies have revealed that the macromolecular carrier is stable in plasma and is resistant to clearance by the reticuloendothelial system. DE-310 showed antitumor activity in various tumor models by single and repeated administration (1), and antitumor activity was noted at lower doses than with DX-8951. The two DE-310 breakdown products, DX-8951 and glycyl-DX-8951, were found to exert antitumor activity *in vivo* (6), whereas the CYP3A4- and CYP1A2-mediated DX-8951 metabolites referred to as UM-1 and UM-2 were less potent (7–10).

Previous phase I clinical studies with various administration schedules of DX-8951f have shown reversible, noncumulative, and dose-dependent neutropenia and sometimes thrombocytopenia (11–16). Other side effects included mild to moderate gastrointestinal toxicity (nausea, vomiting, stomatitis, and diarrhea), fatigue, asthenia, and alopecia (11–16). Reversible, noncumulative, neutropenia was the dose-limiting toxicity (DLT) in all schedules (11–15), as well as transient and reversible liver dysfunction (12, 14), or stomatitis in advanced leukemia (16).

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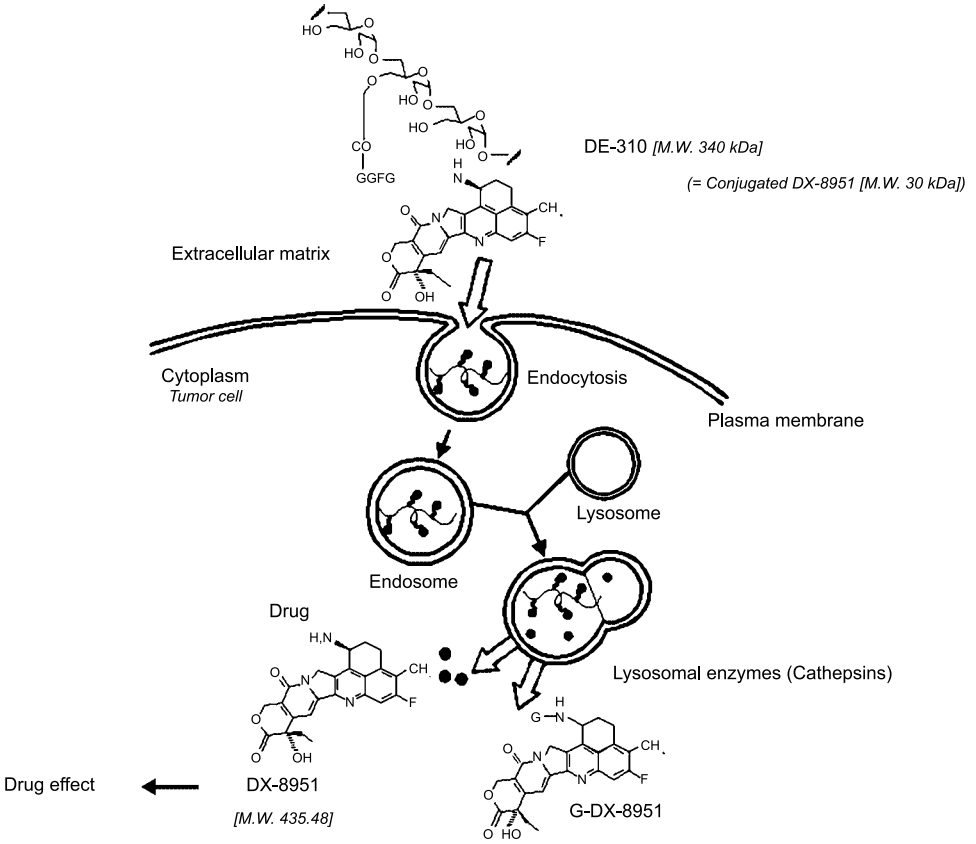

Fig. 1 Schematic representation of intracellular drug release by cleavage of the peptidyl linker by lysosomal enzymes. After internalization of the macromolecular carrier DE-310 (*MW,* 3.4×10^5) by endocytosis, the macromolecule is transferred to the lysosomal compartment, where it is exposed to lysosomal enzymes (i.e., cathepsin B and cathepsin L). Both glycyl-DX-8951 and free DX-8951 (*MW,* 435.48) are liberated intracellularly. The biodegradable polymer is metabolized and afterwards excreted renally.

The objectives of this phase I study were (a) to determine the maximum-tolerated dose and dose-limiting toxicities (DLTs) of DE-310 when given i.v. once every 2 or 6 weeks, (b) to characterize the pharmacokinetics of DE-310 (as conjugated DX-8951) and its metabolites DX-8951 and glycyl-DX-8951, and (c) to evaluate preliminary antitumor activity.

PATIENTS AND METHODS

Eligibility Criteria. Patients with a histologically confirmed diagnosis of a malignant solid tumor refractory to conventional chemotherapy or for whom no effective therapy existed were eligible. Other eligibility criteria included the following: age ≥ 18 years; Eastern Cooperative Oncology Group performance status 0 to 2; estimated life expectancy of ≥8 weeks; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosoureas, mitomycin C, or carboplatin); and adequate hematopoietic [absolute WBC count > 3,000/μL, absolute neutrophil count > 1,500/μL, platelet count > 100×10^9/L, and hemoglobin > 8.5 g/dL (or 5.2 mmol/L)], hepatic [serum total bilirubin < 1.5 mg/dL (25.6 μmol/L), serum aspartate transaminase, and alanine transaminase < 2.5 times the institutional upper normal limit (<5.0 times upper normal limit in case of liver metastases)], and renal (serum creatinine concentration < 1.5 mg/dL or if raised between 1.5 and 2.0 mg/dL, then a creatinine clearance of >60 mL/min) function. Specific exclusion criteria included symptomatic brain metastases, active or uncontrolled infection, known allergy to camptothecin derivatives, concomitant treatment with CYP 3A4 inhibitors or inducers (wash-out period of at least 7 days since last intake), or diarrhea (>2-3 stools/d above normal frequency during the past 4 weeks). The institutional Ethical Board approved the study protocol. All patients gave written informed consent before study entry.

Treatment and Dose Escalation. DE-310 for injection (37.9 mg of conjugated DX-8951 (i.e., equivalent to 2.5 mg of DX-8951) and 165 mg maltose-monohydrate per 5 mL vial) was provided as a lyophilized powder, and was stored in a refrigerator at 2 to 8°C and protected from light in a closed package. The drug was supplied by Daichii Pharmaceutical Co., Ltd. (Tokyo, Japan). DE-310 was reconstituted with 5 mL of 0.9% sodium chloride to obtain a concentration of 0.5 mg DX-8951 equivalent/mL solution and then filtered. The appropriate volume of stock solution needed to yield the required dose was diluted in a polyvinylchloride infusion bag with 0.9% sodium chloride to 250 mL. This dilution was stable for at least 24 hours when protected from light. The diluted drug product was given protected from light over a period of 3 hours, using a programmed peristaltic pump system. With the exception of the first and second administration of the first and second cycle of the q2w schedule and the first and second cycle of the q6w schedule, respectively, in which patients were hospitalized for pharmacokinetic blood sampling, patients were treated on an outpatient basis. Prophylactic therapy was prohibited for the first administration, but could be given from the second

administration if treatment was delayed (>24 hours) or when constant nausea and vomiting occurred. Prophylactic growth factors were not allowed.

The starting dose of DE-310 was 1.0 mg/m^2, and each infusion was followed by a 2 weeks recovery period for every 28 days of treatment (q2w schedule) and was based on 1/6 of the highest nonsevere toxic dose found in the dog. In the q2w schedule, cycles were repeated every 28 days. After protocol amendment to a six-weekly schedule, based upon available information from a parallel phase I study using an every 4 weeks schedule (17), the dose of DE 310 was escalated to 6.0 mg/m^2, followed by a 6-week interval (q6w schedule). Further dose escalations were based on the prior dose level toxicity.

At least three patients were entered at each dose level. If one of three patients experienced DLT, three additional patients were to be entered at that dose level. The maximum-tolerated dose was defined as one dose level below the dose that induced DLTs in at least 2 out of 6 patients, defined as the National Cancer Institute Common Toxicity Criteria version 2.0 grade 4 neutropenia lasting for $\geq$5 days, neutropenic fever (defined as grade 4 neutropenia with fever $\geq$ 38.5°C), thrombocytopenia $\leq 25 \times 10^9$/L, any other clinically significant grade 4 hematologic toxicity, grade 3 or 4 vomiting despite maximum supportive care, any other grade 3 or 4 nonhematologic toxicity, and for the q2w schedule inability to start the second administration of the first cycle or to start a second cycle after a 1-week delay because of unresolved toxicity (18). Unresolved toxicity was defined as WBC $< 3.0 \times 10^9$/L, absolute neutrophil count $< 1.5 \times 10^9$/L, platelet count$< 100 \times 10^9$/L, and all associated nonhematologic toxicities (excluding alopecia) not yet recovered to grade 0 to 1. Intrapatient dose escalation was not allowed. The treatment was resumed when the neutrophil count had recovered to $\geq 1.5 \times 10^9$/L, the platelet count to $\geq 100 \times 10^9$/L, and any other treatment-related toxicities were $\leq$grade 1.

Treatment Assessment. Before initiating therapy, a complete medical history was taken and a physical examination was done. A complete blood cell count and serum biochemistry were done, as were ECG, urinalysis, and chest X-ray. Weekly evaluations included history, physical examination, toxicity assessment according to the National Cancer Institute Common Toxicity Criteria, serum biochemistry, and urinalysis. Complete blood cell counts were determined twice weekly throughout every cycle. Tumor evaluation was done after every two cycles in the q2w schedule, and after every cycle in the q6w schedule, respectively, according to RECIST (19).

Sample Collection and Drug Analysis. For pharmacokinetic analysis, a total of 40 blood samples (~6 mL each) over a period of 8 weeks for the q2w schedule and over a period of 12 weeks for the q6w schedule, respectively, was obtained from an indwelling i.v. canula and collected into a heparin-containing tube. In the q2w schedule, samples were taken immediately prior to drug administration of DE-310, at the end of infusion, and at 1, 2, 4, 6, 8, 24, 48, 96, and 144 hours post-end of infusion. In the q6w schedule, samples were taken immediately prior to drug administration of DE-310, and at 1, 2, 4, 6, 8, 24, 48, 72, 168, 240, 336, 504, 672, and 840 hours post-end of infusion. Blood specimens were immediately put in an ice-water bath (4°C) until centrifugation at 2,000 rpm for 15 minutes at 4°C. Plasma was transferred into plastic specimen storage vials and stored frozen at −20°C until analysis. Also, urine was collected for pharmacokinetic analysis over a 24-hour period on days 1 to 8 in the first two cycles of the q2w schedule and over a 24-hour period on days 1 to 2, and over a 12-hour period on days 3, 7, 14, 21, 28, 35, and 42 in the first two cycles of the q6w schedule. Urine samples were stored frozen at −20°C until analysis.

The plasma concentrations of conjugated DX-8951, glycyl-DX-8951 and DX-8951 were quantified by a validated liquid chromatographic method (MDS Pharma Services, Montreal, Canada). Concentrations of conjugated DX-8951 were determined using a thermolysin enzymatic reaction and fluorescence detection.

Samples of saliva and erythrocytes, normal skin, and superficial tumor tissue, if possible, were obtained from patients at the dose levels 6.0 and 7.5 mg/m^2. Saliva was obtained during the first cycle prior to drug administration, at the end of infusion, 4 hours post-end of infusion, and once a day on days 2, 3, 4, 8, 11, 15, 22, 29, and 36. Samples of erythrocytes were collected at the time points indicated for plasma. The concentrations of conjugated DX-8951, glycyl-DX-8951, and DX-8951 in saliva, erythrocytes and ascites were quantified by a validated method as described previously (20). Normal skin and tumor tissue was obtained on day 8 or 9 of the first cycle and analyzed as described for plasma (Shin-Nippon Biomedical Laboratory Ltd., Kainan, Japan).

Pharmacokinetic and Pharmacodynamic Data Analysis. Pharmacokinetic variables were calculated by standard non-compartmental methods using WinNonlin software version 3.3 (Pharsight, Mountain View, CA), and included time to peak concentration (T_{max}), peak concentration (C_{max}), terminal half-life ($T_{1/2}$), and area under the curve (AUC). Urine excretion was calculated in percentage of the dose given. Pharmacokinetic variables are provided as mean values with the percent coefficient of variation, and the level of significance (P) for statistical tests was set at 0.05.

RESULTS

Patients and Treatment. A total of 27 patients (16 males and 11 females) with a median age of 57 years was enrolled into the study between February 2001 and February 2003 (Table 1). All patients were eligible and assessable for toxicity. A total of 61 cycles (i.e., 86 administrations) of treatment was given at dose levels of 1.0 mg/m^2 (q2w), 2.0 mg/m^2 (q2w), 6.0 mg/m^2 (q6w), 7.5 mg/m^2 (q6w), and 9.0 mg/m^2 (q6w). Twenty-five patients were assessable for response; one patient died due to massive pulmonary embolism before scheduled tumor reassessment, and another patient withdrew because of rapid disease progression and was replaced (i.e., dose level, 6.0 mg/m^2).

Dose-Limiting Toxicity. At the starting dose of 1.0 mg/m^2, the cohort was expanded to six patients because of the initial classification of DLT due to prolonged grade 1 thrombocytopenia in one patient, delaying the second administration of DE-310 of the first cycle. Mild thrombocytopenia, in retrospect, was related to cirrhosis and esophageal varices resulting in occult gastrointestinal bleeding. No further DLTs were observed with this schedule. Because pharmacokinetics revealed a drug terminal half-life

Table 1 Patient characteristics

Characteristic		No. patients
No. patients		
Total		27
Assessment		
For dose-limiting toxicity		27
For efficacy		25
Gender, male to female	16:11	
Age, y		
Median	57	
Range	31-78	
ECOG performance status		
0		7
1		19
2		1
Previous therapy		
Chemotherapy only		19
Median no. regimens	2	
Range	1-4	
Radiotherapy		2
Both		3
None		3
Tumor types*		
Gastrointestinal tract, including:		12
Colorectal cancer		6
Esophageal cancer		3
Pancreatic cancer		3
Melanoma		5
Lung cancer (NSCLC)		3
Soft tissue sarcoma		3
Unknown primary adenocarcinoma		2
Miscellaneous		4

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NSCLC, non–small cell lung carcinoma.

*Two patients had double tumors.

exceeding the 2 weeks administration interval, the protocol was amended to a 6-week interval between administrations.

The initial three patients at the q6w schedule were treated at a dose of 6.0 mg/m^2 and experienced no DLTs. Subsequently, a dose of 9.0 mg/m^2 was explored, where two patients developed DLT. A female patient with metastatic pancreatic cancer had grade 4 leukocytopenia and grade 4 neutropenia with fever on day 8, as well as grade 3 thrombocytopenia, grade 2 anemia, and grade 3 hepatotoxicity (elevated transaminases). All side effects were reversible. The second and third cycles were given at a dose of 6.0 mg/m^2 and were uneventful. A second female patient with metastatic adenocarcinoma of unknown primary had an uncomplicated first cycle, but on day 11 of her second cycle she developed grade 3 thrombocytopenia, grade 3 neutropenia, grade 2 anemia, grade 3 hepatotoxicity (i.e., hyperbilirubinemia and elevated transaminases). An ultrasound guided liver biopsy revealed veno-occlusive liver disease (VOD). Hyperbilirubinemia persisted for 7 weeks and subsequently recovered but no further DE-310 was given. In view of the DLTs observed at 9.0 mg/m^2, the cohort of 6.0 mg/m^2 was further expanded with three additional patients, none of whom experienced DLTs. A total of 17 cycles were given at dose level 6.0 mg/m^2.

Subsequently, an intermediate dose of 7.5 mg/m^2 was explored. At this dose level, a total of 10 cycles were given, one out of six patients developed DLT consisting of grade 3 hepatotoxicity (i.e., hyperbilirubinemia and elevated transaminases) occurring on day 15 of the second cycle. An ultrasound guided liver biopsy again revealed VOD, which was fully reversible within 15 weeks. The recommended dose for phase II trials of DE-310 was set at 7.5 mg/m^2 once every 6 weeks.

Hematologic and Nonhematologic Toxicity. A summary of the worst grade hematologic toxicities per patient is provided in Table 2. In addition to the dose-limiting toxicities, the other observed nonhematologic toxicities were mild to moderate and consisted of nausea, vomiting, stomatitis, anorexia, asthenia, and alopecia (Table 3). Fifty-one percent of patients experienced short lasting nausea, and 37% experienced incidental vomiting. Prophylactic antiemetics were not given. Reversible increase of the liver transaminases was encountered in 12 out of 27 patients (44%) across all dose levels, except 1.0 mg/m^2 (Table 4).

Pharmacokinetics. Pharmacokinetic analysis was done in all patients. Across the various dose levels, the apparent terminal half-lives of conjugated DX-8951, glycyl-DX-8951, and DX-8951 were 13, 12, and 13 days, respectively (Tables 5 and 6). Consequently, plasma concentrations of all compounds conjugated were sustained for several weeks. This parallel decline suggests that the terminal phase seen with the DE-310 metabolites (i.e., glycyl-DX-8951 and DX-8951) is not due to real elimination processes but due to their much slower formation from conjugated DX-8951. At the recommended dose of 7.5 mg/m^2, substantial interindividual variability was observed (coefficient of variation, up to 92%). The AUC ratio of conjugated DX-8951 to DX-8951 was $\sim$600, a similar value seen as predicted by preclinical studies (1). The AUCs of conjugated DX-8951, glycyl-DX-8951, and DX-8951 seemed to increase in near proportion with an increase in dose.

Table 2 Hematological toxicity (worst grade per patient)

				Anemia		Leukocytopenia				Neutropenia				Thrombocytopenia		
				Grades												
Dose (mg/m^2)	No. patients	No. cycles	No. administrations	1	2	1	2	3	4	1	2	3	4	1	2	3
Every 2 weeks																
1.0	6	13	24	0	1	0	0	0	0	0	0	0	0	2	0	0
2.0	4	16	30	0	2	0	0	0	0	0	0	0	0	0	0	0
Every 6 weeks																
6.0	7	17	17	1	2	2	0	0	0	2	0	0	0	1	0	0
7.5	6	10	10	2	3	0	1	0	0	0	0	1	0	0	1	1
9.0	4	5	5	0	2	2	1	0	1*	2	0	1	1*	0	0	2*

*Considered DLTs in two patients at dose level 9.0 mg/m^2 every 6 weeks in the first and second cycle, respectively.

Table 3 Nonhematological toxicity (worst grade per patient)

				Nausea		Vomiting		Stomatitis		Anorexia		Fatigue		Alopecia	
				Grades											
Dose (mg/m^2)	No. patients	No. cycles	No. administrations	1	2	1	2	1	2	1	2	1	2	1	2
Every 2 weeks															
1.0	6	13	24	1	0	1	0	0	0	0	0	2	1	0	0
2.0	4	16	30	2	1	2	1	0	0	0	0	1	2	1	0
Every 6 weeks															
6.0	7	17	17	5	0	0	3	2	1	3	1	5	2	1	0
7.5	6	10	10	2	1	0	1	1	0	3	0	6	0	1	0
9.0	4	5	5	2	0	2	0	0	0	1	0	2	1	1	0

Urinary excretion was very slow, and total drug recovery over the entire sampling period amounted to <1%. In line with the small distribution volume of conjugated DX-8951, concentrations of conjugated DX-8951, glycyl-DX-8951, and DX-8951 were not detectable in erythrocytes and saliva, further pointing to limited distribution outside the plasma compartment. In tumor biopsies of four patients, concentrations of both glycyl-DX-8951 and DX-8951 were detectable at low levels, whereas in normal skin biopsies of nine patients concentrations of DX-8951 remained below the detection limit (Table 7). No drug levels were obtained on the hepatic tissues of two patients taken at the ultrasound guided liver biopsies.

During the first and second cycle at 6.0 mg/m^2, the concentrations were also assessed in ascites of a patient with metastastic pancreatic cancer (Table 8).

Efficacy. A histologically proven complete remission, confirmed by surgery and still persisting at a follow-up duration of >2 years, was documented in the 56-year-old female with lymph node metastases of adenocarcinoma of unknown primary, who experienced DLT at dose level 9.0 mg/m^2 and only received two cycles.

A partial response lasting 3 months was achieved in a 46-year-old female with liver metastases from pancreatic cancer initially treated at 9.0 mg/m^2 during the first cycle and treated at 6.0 mg/m^2 during the second and third cycle. Whereas according to actual measurements, another partial remission of liver metastases was seen in a 58-year-old female with metastatic ovarian cancer, treated at 2.0 mg/m^2 for seven cycles, we reassessed this response to stable disease since the liver metastases were small and difficult to measure. The serum levels of the tumor marker CA 125 in this patient has decreased from 1,079 to 457 kilounits/L. Progression occurred in this patient after 8 months. A total of 14 patients showed disease stabilization for 6 weeks (n = 1, fibrous histiocytoma), 8 weeks (n = 1, colorectal cancer), 10 weeks (n = 1, colorectal cancer), 12 weeks [n = 5, pancreatic cancer (n = 2); colorectal cancer, adenocarcinoma of unknown primary, and melanoma (each n = 1)], 16 weeks (n = 1, urothelial cancer), 18 weeks (n = 2, non–small cell lung carcinoma and leiomyosarcoma uteri), 24 weeks (n = 2, colorectal cancer and alveolar soft part sarcoma), and 32 weeks (n = 1, ovarian cancer).

DISCUSSION

We did this study to assess the safety, tolerability, maximum-tolerated dose, and pharmacokinetics of DE-310, a macromolecular prodrug of the topoisomerase I inhibitor exatecan (DX-8951f), given as a 3-hour i.v. infusion once every 2 or 6 weeks.

The present study showed that prolonged concentrations of DX-8951 were achieved by only single short infusion of DE-310 given every 6 weeks, which confirmed the pharmacokinetic proof of principle yielding the extended exposure to the topoisomerase I inhibiting active moiety.

Because of the long half-life, which approximated a 2-week administration interval and largely exceeded the half-life expected based on preclinical data, further exploration of the q2w schedule was considered illogical in view of the potential for drug accumulation, and therefore the study protocol was

Table 4 Hepatotoxicity (worst grade per patient)

				ALT			AST			Total bilirubin		
				Grades								
Dose (mg/m^2)	No. patients	No. cycles	No. administrations	1	2	3	1	2	3	1	2	3
Every 2 weeks												
1.0	6	13	24	0	0	0	0	0	0	0	0	0
2.0	4	16	30	0	1	0	1	1	0	1	0	0
Every 6 weeks												
6.0	7	17	17	2	0	0	4	0	0	0	0	0
7.5	6	10	10	0	2	1*	0	1	2*	1	1	1*
9.0	4	5	5	1	0	2†	1	1	2†	0	0	1*

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase.

*Considered a DLT in one patient at dose level 7.5 mg/m^2 every 6 weeks, and in one patient at dose level 9.0 mg/m^2 every 6 weeks in the second cycle.

†Considered a DLT in one patient at dose level 9.0 mg/m^2 every 6 weeks in the first cycle.

Table 5 Mean (CV%) pharmacokinetic parameters of conjugated DX-8951, glycyl-DX-8951, and DX-8951 of first administration at first course in every 2-week schedule and at first course in every 6-week schedule

		Conjugated DX-8951					Glycyl-DX-8951					DX-8951				
Dose (mg/m²)		$AUC_{0\text{-}t}$ (ng h/mL)	$AUC_{0\text{-}336\ h}$ (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0\text{-}t}$ (ng h/mL)	$AUC_{0\text{-}336\ h}$ (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0\text{-}t}$ (ng h/mL)	$AUC_{0\text{-}336\ h}$ (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)
Every 2 weeks																
1.0	Mean	38,402.0*	n/a	460.8	5.3	n/a	238.5	n/a	1.54	132.0	n/a	158.6	n/a	0.87	135.0	n/a
(n = 6)	CV%	67.7		53.8	47.6		67.8		71.7	51.9		72.5		71.7	44.6	
2.0	Mean	61104.0†	n/a	582.6†	4.0†	n/a	515.8‡	n/a	2.23†	99.0*	n/a	366.6‡	n/a	1.6	87.0	n/a
(n = 4)	CV%	n/a		n/a	n/a		130.2		123.7	39.6		111.7		100.7	52.8	
Every 6 weeks																
6.0	Mean	661,924.4	498,855§	3,344.7	5.0	243.4	1,731.2	929.2§	6.71	251.0	348.4	960.4	642.8§	3.2	150.4	260.8
(n = 7)	CV%	45.5	36.0	17.5	38.3	40.8	55.5	60.0	93.3	78.9	47.0	39.2	34.7	56.6	50.8	21.1
7.5	Mean	1,124,472.3	793,120.2	5,706.3	4.7	338.8	3,907.2	2,492.4	11.6	123.0	330.2	1781.1	1168.1	5.2	151.0	302.0
(n = 6)	CV%	19.4	20.1	18.9	50.1	18.3	90.1	92.6	93.7	42.7	78.5	78.9	83.6	84.5	68.5	54.0
9.0	Mean	902,743.5	629,527.3	4,218.9	5.5	309.5	3,178.7	2,299.6	12.6	183.0	186.3	2062.8	1479.0	6.9	135.0	169.5
(n = 4)	CV%	60.3	56.5	33.8	54.5	37.3	71.5	89.9	118.0	71.4	16.2	53.2	56.3	66.8	65.7	18.4

NOTE. $AUC_{0\text{-}t}$: AUC calculated from time 0 to the last nonzero concentration; $AUC_{0\text{-}336h}$: AUC calculated from time 0 to the concentration at time 336 hours post-end of infusion, where possible. Because the distribution phase of glycyl-DX-8951 and DX-8951f from conjugated DX-8951 and the glycyl-DX-8951 and DX-8951 elimination phase could be simultaneously occurring over the dosing interval, a true terminal elimination phase cannot be accurately characterized using noncompartimental methods. An apparent elimination phase half-life ($T_{1/2,app.}$) is therefore reported here.

Abbreviations: CV, coefficient of variation; C_{max}, peak plasma concentration; T_{max}, time to C_{max}; $T_{1/2}$, half-life of terminal phase; n/a, not available.

*Calculated on data from four patients.

†Calculated on data from one patient.

‡Calculated data from three patients.

§Calculated on data from six patients.

amended to a 6 weeks schedule based on available information from a parallel trial using an every 4 weeks schedule (17). The pharmacokinetics showed near dose proportionality of conjugated DX-8951, glycyl-DX-8951, and DX-8951.

Reversible grade 4 myelosuppression and grade 3 hepatotoxicity with VOD were the principal DLTs and were observed at dose levels 9.0 mg/m² (2 out of 4 patients) and 7.5 mg/m² (1 out of 6 patients), respectively. It is of interest to

Table 6 Mean (CV%) pharmacokinetic parameters of conjugated DX-8951, glycyl-DX-8951, and DX-8951 of second administration at first course in every 2-week schedule and at second course in every 6-week schedule

		Conjugated DX-8951					Glycyl-DX-8951					DX-8951				
Dose (mg/m²)		$AUC_{0\text{-}t}$ (ng h/mL)	$AUC_{0\text{-}336\ h}$ (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0\text{-}t}$ (ng h/mL)	$AUC_{0\text{-}336\ h}$ (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0\text{-}t}$ (ng h/mL)	$AUC_{0\text{-}336h}$ (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)
Every 2 weeks																
1.0	Mean	31,256.5	n/a	324.9	3.8	n/a	321.7	n/a	3.8	36.5	n/a	321.8	n/a	4.9	51.0	n/a
(n = 4)	CV%	114.8		72.9	25.5		111.5		134.4	116.3		66.7		98.5	66.6	
2.0	Mean	221,846.0	n/a	1,230.3	3.5	n/a	834.5*	n/a	4.0*	292*	n/a	631.0*	n/a	3.1*	326*	n/a
(n = 4)	CV%	75.1		42.8	16.5		93.9		112.7	143.1		86.2		115.7	119.9	
Every 6 weeks																
6.0	Mean	750,514.6	519,098.0	3,541.4	6.0	295.8	2,989.8	1,540.0	7.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a
(n = 5)	CV%	32.6	25.6	22.0	47.1	19.7	100.4	72.4	98.4	n/a	n/a	n/a	n/a	n/a	n/a	n/a
7.5	Mean	1,180,980.0	813,841.0	5,081.0	6.3	418.3	1,3080.3	6,324.2	33.4	n/a	n/a	n/a	n/a	n/a	n/a	n/a
(n = 3)	CV%	42.7	39.0	13.2	63.8	38.2	102.8	98.0	110.9	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9.0	Mean	1,313,751.0	920,467.3	4,868.5	15.0	267.0	13,893‡	3,814.9‡	36.5‡	339‡	616‡	7039.3‡	2811.5‡	19.8‡	339‡	181‡
(n = 2)†	CV%	71.7	73.8	55.7	113.1	4.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

NOTE. $AUC_{0\text{-}t}$: AUC calculated from time 0 to the last nonzero concentration; $AUC_{0\text{-}336\ h}$: AUC calculated from time 0 to the concentration at time 336 hours post-end of infusion, where possible. Because the distribution phase of glycyl-DX-8951 and DX-8951f from conjugated DX-8951 and the glycyl-DX-8951 and DX-8951 elimination phase could be simultaneously occurring over the dosing interval, a true terminal elimination phase cannot be accurately characterized using noncompartimental methods. An apparent elimination phase half-life ($T_{1/2,app.}$) is therefore reported here.

Abbreviations: CV, coefficient of variation; C_{max}, peak plasma concentration; T_{max}, time to C_{max}; $T_{1/2}$, half-life of terminal phase; n/a, not available.

*Calculated on data from three patients.

†Dose reduction from 9.0 to 6.0 mg/m² (every 6 weeks) for one patient in course 2.

‡Calculated on data from one patient.

Table 7 Concentrations (ng/g) of DE-310 (as conjugated DX-8951), G-DX-8951 and DX-8951 in normal skin tissues (normal) and tumor biopsies (tumor)

Patient (tumor type)	Dose,* (mg/m^2)	Time of biopsy at first cycle (d)	Normal versus tumor	Biopsy of tumor type	Weight of sample (mg)	Conjugated DX-8951	G-DX-8951	DX-8951
19 (sarcoma)	6.0	8	Normal		5.8	BLQ	BLQ	BLQ
20 (colorectal)	6.0	8	Normal		9.8	BLQ	62.731	BLQ
21 (melanoma)	6.0	9	Normal		32.7	BLQ	24.524	BLQ
			Tumor	Melanoma	153.5	166.159	134.457	8.037
22 (melanoma)	7.5	9	Normal		18.9	BLQ	53.448	BLQ
			Tumor	Melanoma	2,294.1	303.557	155.276	4.188
23 (melanoma)	7.5	8	Normal		15.9	BLQ	59.173	BLQ
			Tumor	Melanoma	2,223.0	222.019	119.103	4.157
24 (NSCLC)	7.5	8	Normal		9.3	BLQ	10.870	BLQ
25 (ACUP)	7.5	9	Normal		9.2	BLQ	5.617	BLQ
26 (sarcoma)	7.5	8	Normal		27.2	BLQ	8.419	BLQ
		9	Tumor	Sarcoma	414.2	160.690	54.202	2.159
27 (melanoma)	7.5	8	Normal		29.1	BLQ	10.199	6.613

Abbreviations: G-DX-8951, glycyl-DX-8951; BLQ, below limit of quantitation; NSCLC, non–small cell lung cancer; ACUP, adenocarcinoma unknown primary.

*Every 6 weeks.

note that the AUCs of conjugated DX-8951, glycyl-DX-8951, and DX-8951 were 10-fold higher in the second cycle of the two patients with resulting VOD compared with the other patients at the same dose levels. The reason for this large interpatient variation in pharmacokinetics remains to be elucidated. The recommended dose for phase II studies is 7.5 mg/m^2 once every 6 weeks. At this dose and schedule, circulating concentration of the active moiety DX-8951 after a single infusion of DE-310 were similar to those achieved at the maximum-tolerated dose of a 21-day continuous infusion of DX-8951f at 0.15 mg per m^2 per day (Fig. 2; ref. 21). The number of patients with multiple courses is limited in this study. Thus, we can not fully exclude the possibility of toxicity being cumulative and therefore also a longer cycle interval or a lower dose of 6 mg/m^2 every 6 weeks may be considered for future studies. Because we were unable to study this, it can not yet be completely excluded that the polymer accumulates in the liver and thereby generates liver damage.

Other nonhematalogic toxicities attributed to the treatment of DE-310 included mild to moderate nausea, vomiting, stomatitis, anorexia, asthenia, and alopecia, a toxicity profile similar to that of single agent exatecan mesylate (DX-8951f; refs. 11–15).

The observed myelosuppression was an expected side effect, known to be a class effect of topoisomerase I inhibitors. The nadirs of absolute neutrophil count and platelets were reached at 22 days (range, 18-29 for absolute neutrophil count and 11-36 for platelets) post-infusion. Myelosuppression was always fully reversible after a median of 14 days (range, 3-23) post-nadir. In view of the presence of circulating active drug moiety at the time of nadir and the conceptual likelihood that colony-stimulating growth factors could thus actually increase severity and duration of myelosuppression by stimulating stem cells, whereas exposed to cytotoxics, the administration of agents like granulocyte colony-stimulating factor was specifically avoided.

The other DLT was hepatotoxicity consisting of reversible increases in liver transaminases and bilirubin, and in two patients correlated with presence of hepatic VOD. Hepatic VOD results from toxic injuries to the hepatic sinusoids (zone 3 of the liver acinus), followed by a series of biological processes that lead to circulatory compromise of centrilobular hepatocytes, fibrosis, and obstruction of liver blood flow

Table 8 Concentrations (ng/mL) of DE-310 (as conjugated DX-8951), G-DX-8951, and DX-8951 in plasma and ascites in one patient with metastatic pancreatic cancer (dose, 6.0 mg/m^2, every 6 weeks)

		Plasma			Ascites		
Cycle	Day	Conjugated DX-8951	G-DX-8951	DX-8951	Conjugated DX-8951	G-DX-8951	DX-8951
1	1	2,987.9*	2.333†	0.545†	nd	nd	nd
	15	424.3	1.974	0.448	1,401.97	3.66	0.61
	29	119.3	0.610	0.149	382.18	0.46	0.11
	36	BLQ	0.352	0.084	232.82	BLQ	BLQ
	39	nd	nd	nd	197.51	BLQ	BLQ
2	1	4,067.1‡	2.643†	0.785†	172.49	BLQ	BLQ
	4	1,759.9	7.912	1.578	3,164.66	6.74	0.67
	18	nd	nd	nd	572.32	1.64	0.21
	22	305.9	0.948	0.181	466.62	1.10	0.18
	30	BLQ	0.321	0.073	103.1	BLQ	BLQ

Abbreviations: G-DX-8951, glycyl-DX-8951; nd, not determined; BLQ, below limit of quantitation (for ascites <0.5 ng/mL).

*Maximum concentration at 4 hours after end of infusion.

†Maximum concentration at 24 hours after end of infusion.

‡Maximum concentration at 2 hours after end of infusion.

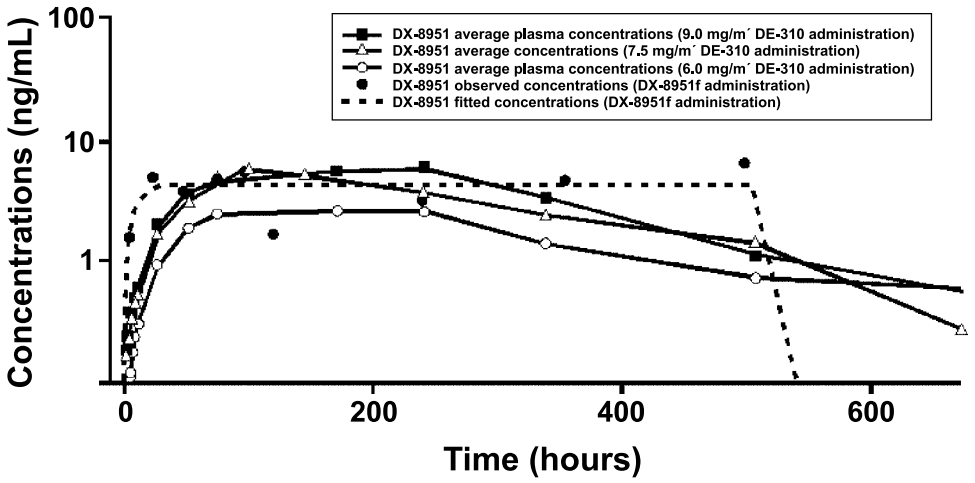

Fig. 2 Comparative exposure of 21-day continuous infusion of 0.15 mg per m^2 per day of DX-8951f (exatecan; ref. 21) *versus* single 3-hour infusions of increasing doses of DE-310.

(22, 23). Histology showed diffuse damage in the centrilobular zone of the liver, in combination with sinusoidal fibrosis, necrosis of pericentral hepatocytes, narrowing, and fibrosis of central veins. Although drug-induced VOD is most frequently observed following stem cell transplantations with high-dose busulfan and cyclofosfamide, it can occur after various regimens of chemotherapy at conventional doses (e.g., actinomycin D, dacarbazine, cytosine arabinoside, mitramycin, and 6-thioguanine), combination chemotherapy, or (total body) irradiation. It has been suggested that drugs or toxins can induce depletion of glutathione leading to cell death and yielding increased activity of matrix metalloproteinases, resulting in degradation of the extracellular matrix and loss of sinusoidal endothelial cells from the space of Disse (22, 23).

Hepatotoxicity was also reported in a phase I study of *N*-(2-hydroxypropyl)-methacrylamide copolymer conjugates of doxorubicine (24) but not for MAG-camptothecin (25), and the phase I study of pegylated camptothecin (26). It therefore remains unclear if the carrier is responsible for this phenomenon, or whether both carrier and active drug are responsible. Whereas VOD is a severe side effect, the fact that even the most severe cases of hepatotoxicity in our trial showed full reversibility indicates that with proper monitoring the drug DE-310 can be safely used at the recommended dose of 7.5 mg/m^2 every 6 weeks.

Concentrations of conjugated DX-8951, glycyl-DX-8951, and DX-8951 were determined in ascites samples in a patient with advanced pancreatic disease treated at dose level 6.0 mg/m^2. These data indicated significant exposure of the peritoneal cavity to conjugated DX-8951, glycyl-DX-8951, and DX-8951 at concentrations exceeding those in simultaneously obtained plasma samples.

The results of studies assessing DE-310, glycyl-DX-8951, and DX-8951 in normal skin, erythrocytes, and saliva would seem to fit with the long circulation times combined with the formation rate-limited elimination and the hypothesis of preferential drug uptake by tumor tissue and not in other tissues. However, the sample size and available data are too limited to draw definitive conclusions. Notwithstanding, it can be concluded that the biodistribution of this polymerized agent is substantially different from that of the registered topoisomerase I inhibitors topotecan (27) and irinotecan and its metabolite SN-38 (28).

In summary, by employing a polyalcohol carrier with cleavable linking peptide as a drug delivery system, the pharmacokinetics profile of topoisomerase I inhibitor DX-8951f seems to be improved, providing slow release of the active moiety over a very extended period. This together with the presence of discernable drug levels in tumor and the below limits of quantification drug levels in normal tissues provides supportive evidence to the validity of the concept of using macromolecular carriers to enhance the potential efficacy and diminish the toxicity of topoisomerase I inhibitors and warrants further evaluation in phase II studies.

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