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A phase I clinical and pharmacokinetic study of the dolastatin analogue cemadotin administered as a 5-day continuous intravenous infusion

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Abstract *Purpose*: The dolastatins are a class of naturally occurring cytotoxic peptides which function by inhibiting microtubule assembly and tubulin polymerization. Cemadotin is a synthetic analogue of dolastatin 15 with potent antiproliferative and preclinical antitumor activity. This report describes a phase I study to evaluate the administration of cemadotin to adult cancer patients by a 5-day continuous intravenous (CIV) infusion. *Methods*: All patients had histologically confirmed refractory solid tumors. The dose was escalated from an initial level of 2.5 mg/m^2 (0.5 mg/m^2 daily) according to a modified Fibonacci algorithm. A minimum of three patients was evaluated at each dose level until the maximum tolerated dose (MTD) was established. Treatment was repeated every 21 days until patients were removed from the study due to toxicity or disease progression. Drug-related toxicities were evaluated and graded by the U.S. National Cancer Institute's Common Toxicity Criteria. A radioimmunoassay (RIA) that detected both the parent drug and its metabolites with an intact N-terminal region of the molecule was used for pharmacokinetic studies. *Results*: Twenty heavily pretreated patients received a total of 40 courses of cemadotin over five dose levels ranging from 2.5 to 17.5 mg/m^2. Reversible dose-related neutropenia was the principal dose-limiting toxicity and 12.5 mg/m^2 was established as the MTD. Nonhematologic toxicities attributed to the drug were moderate, and there was no evidence of the cardiovascular toxicity noted in the prior phase I studies of cemadotin given IV as a 5-min injection or 24-h infusion. There were no objective antitumor responses. Time courses of the cemadotin RIA equivalent concentration in whole blood were defined in 14 patients during the first cycle of therapy. The RIA-detectable species exhibited apparent first-order pharmacokinetics across the entire range of doses. The mean ± SD of the observed steady-state blood concentration at the 12.5 mg/m^2 MTD was 282 ± 7 nM ($n = 3$). Blood levels decayed monoexponentially following the end of the infusion, with a mean half-life of 13.2 ± 4.3 h ($n = 14$) in all patients. Mean values ($n = 14$) of the total blood clearance and apparent volume of distribution at steady state were 0.52 ± 0.09 l/h/m^2 and 9.9 ± 3.3 l/m^2, respectively. *Conclusions*: The cardiotoxic effects of cemadotin were completely avoided by administering it as a 120-h CIV infusion. Thus, cardiovascular toxicity appears to be associated with the magnitude of the peak blood levels of the parent drug or its metabolites, whereas myelotoxicity is related to the duration of time that blood levels exceed a threshold concentration. Nevertheless, the data acquired during the extensive clinical experience with cemadotin requires careful examination to assess whether advancing this compound into disease-oriented efficacy studies is merited.

Key words Cemadotin · Dolastatin · Chemotherapy · Clinical trials · Pharmacokinetics

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Introduction

The dolastatins are a family of cytotoxic linear peptides isolated from the Indian Ocean sea hare, *Dolabella auricularia*, which are distinguished by the presence of unusual amino acid residues [1, 2]. Among the known naturally occurring compounds in this class, the antiproliferative effects of dolastatins 10 and 15 have been

most intensively studied. Both compounds interfere with microtubule assembly in vitro by inhibiting tubulin polymerization and β-tubulin-dependent GTP hydrolysis, with dolastatin 10 being approximately 10 times more potent than dolastatin 15 [3, 4]. These interactions block the mitotic progression of proliferating cancer cells, resulting in arrest at the G_2/M phase of the cell cycle and induction of apoptosis [5].

While dolastatin 10 was selected for introduction into clinical trials for evaluation as an anticancer drug [6, 7], dolastatin 15 has primarily served as a lead compound for improving the pharmacologic and pharmaceutical properties of the natural molecule through structural modifications [8]. Replacing the labile C-terminal (S)-dolapyrrolidinone residue of dolastatin 15 with more readily available amides affords derivatives with greater chemical stability and antiproliferative activities. Cemadotin (*N*,*N*-dimethyl-L-valyl-L-valyl-*N*-methyl-L-valyl-L-prolyl-L-prolinebenzylamide hydrochloride; Fig. 1) is a synthetic water-soluble analogue of dolastatin 15 with a benzylamide moiety as the C-terminal subunit [9]. It exhibits in vitro activity against cell lines derived from a variety of human solid tumors, with 50% inhibitory concentrations ranging from 0.2–1 n*M* [9, 10]. The potency of cemadotin against nonleukemic cell lines is comparable to that of dolastatin 10 [11, 12]. Promising in vivo activity has been demonstrated against tumor xenografts implanted in nude mice [13]. The therapeutically optimal dosing regimen was three 30 mg/kg bolus intravenous (IV) injections given once every other day.

The first phase I clinical trial of cemadotin revealed that hypertension and myocardial infarction were the dose-limiting toxicities (DLTs) when the drug was administered to cancer patients as a 5-min IV injection once every 3 weeks [14]. A number of phase I studies were subsequently performed to extensively examine the influence of the treatment schedule and duration of infusion on cardiovascular toxicity [15, 16, 17, 18]. These studies have demonstrated that the cardiovascular effects of cemadotin are highly schedule dependent. Neutropenia was dose limiting for the more highly fractionated dosing schedules or when the duration of infusion was extended to 24 h. While the severity of hypertension and associated cardiac effects has been significantly modulated, hypertension nevertheless occurred at a relatively high frequency at doses that would otherwise be suitable for phase II studies [15, 16].

This report describes the results of a phase I study in which cemadotin was administered as a 5-day continuous intravenous (CIV) infusion. The primary objective of the study was to further evaluate the schedule dependence of the principal toxicities of cemadotin. It was anticipated that the findings derived from this study, together with information obtained from previous clinical studies of the agent, would serve to establish whether the cardiovascular toxicity resulted from total systemic exposure to the drug or brief periods of exposure to the high drug levels produced by shorter duration infusions. We have also compared the dose intensity achieved with the 120-h CIV infusion schedule with the other schedules that could potentially be employed in phase II trials.

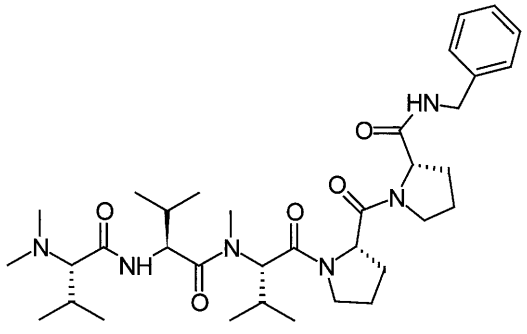

Fig. 1 Chemical structures of cemadotin and *N*-desbenzylamino-cemadotin

Materials and methods

Patient selection

The study was limited to patients with a histologically confirmed diagnosis of a metastatic or inoperable solid tumor for which no potentially curative therapy existed. Patients with brain tumors or clinical indications of CNS involvement were excluded. Patients had to be at least 18 years old with a minimum life expectancy of 12 weeks to facilitate an adequate evaluation of toxicity. The time between major surgery or other prior treatment of the malignancy, including radiotherapy, chemotherapy, and immunotherapy, and entry into the study had to be at least 4 weeks (6 weeks for patients who previously received a nitrosourea or mitomycin C), with full recovery from the effects of any prior treatments. Minimum eligibility requirements of the protocol included the following: Eastern Cooperative Oncology Group performance status ≤2; WBC ≥3500/μl; platelet count ≥100,000/μl; granulocyte count $\geq 2 \times 10^6$/μl; hemoglobin ≥9.0 g/dl; total bilirubin <2.0 mg/dl; serum alkaline phosphatase, glutamic-oxaloacetic transaminase, and glutamic-pyruvic transaminase activities not greater than twice the upper normal limits; normal prothrombin time, serum creatinine ≤1.4 mg/ml or creatinine clearance >60 ml/min; serum glucose ≤200 mg/dl; and serum electrolytes, calcium, and phosphate within normal limits. The requirement for serum alkaline phosphatase and transaminase activities was adjusted to <5 times the upper limit of normal for patients with liver metastases. Occurrence of a significant cardiac or cerebrovascular event within the last year or other serious medical illnesses resulted in the exclusion of patients. A central venous access device was placed if not already present. All patients provided a signed written informed consent that satisfied all federal and institutional requirements.

Dosage and drug administration

Cemadotin (LU 103793) was supplied by Knoll Pharmaceutical Company (Whippany, N.J.) as an injectable solution in 5- and 10-ml ampules containing respectively 250 or 100 mg of the compound as a hydrochloride salt, glycerol, and sodium citrate for pH adjustment to 4–5. The ampules were stored at ambient temperature. Stability of the dosage form following dilution in 0.9% sodium chloride to a concentration of 10 μg/ml or greater has been documented for 7 days at room temperature. The volume of injectable solution which contained 20% of the desired total dose was loaded into a 50-ml medication cassette reservoir (SIMS Deltec, St. Paul, Minn.) and diluted with 0.9% sodium chloride USP such that the final drug concentration remained above 10 μg/ml. CADD-1 Model 5100 or CADD-Plus Model 5400 programmable ambulatory infusion pumps (SIMS Deltec) were used to deliver the dosing solution without an in-line filter. New medication cassettes containing freshly prepared dosing solutions were placed in the pump every 24 h.

Treatment protocol

The study protocol was approved by the institutional Scientific Review Committee and Human Protection Committee. Prospective patients underwent a physical, radiographic, and/or sonographic evaluation to completely stage their tumor within 21 days before entering the study. Additional tests including a complete physical examination and performance status assessment, electrocardiogram, chest X-ray, hematologic, coagulation, and clinical chemistry determinations, and urinalysis were completed within 14 days prior to treatment. Electrocardiograms were obtained daily during the first 120-h infusion of drug, and before and after the administration of each subsequent cycle of therapy. Supine blood pressure, pulse, and physical signs were monitored daily during the infusion for all cycles of therapy. Hematologic values, a clinical chemistry profile, and urinalysis were obtained weekly for all patients while on the study, with a patient history and complete physical examination performed prior to each cycle of therapy or upon disease progression. Concurrent use of any other cytotoxic or investigational antitumor therapy, palliative radiation therapy, and growth factors was prohibited during participation in the study. Prophylactic use of antiemetic medications was not permitted during the initial cycle of therapy unless nausea/vomiting became a major toxicity during the course of the study.

Cemadotin was administered as a 120-h CIV infusion through a central venous catheter. The dose was escalated from an initial level of 2.5 mg/m^2, 0.5 mg/m^2 daily (1/10th of the LD_{10} in mice, 258 mg/m^2) according to a modified Fibonacci progression [19]. Three patients were entered at each dose level with treatment repeated every 3 weeks as permitted by their condition. Evaluation of successive dose levels proceeded after all three patients received the first cycle of therapy with the aforementioned dose and each was observed for at least 21 days without evidence of a DLT, as defined below. An additional three patients were entered into a given dose level in cases where a single patient experienced a DLT during the first cycle of therapy. The occurrence of a DLT in two patients from any cohort of three to six established the preceding dose level as the maximum tolerated dose (MTD). The tentative MTD was expanded by adding three additional patients to better evaluate the toxicity profile of the prospective phase II dose. Drug-related toxicities were evaluated during each cycle of therapy and graded according to the Common Toxicity Criteria developed by the U.S. National Cancer Institute [20]. In a departure from the standard definition of grade 4 hematologic toxicity, a duration of >5 days of grade 4 hematologic toxicity (absolute neutrophil count <500/μl or platelets <25,000/μl) was required before DLT occurred.

Patients were scheduled to receive at least two courses of therapy with the same dose of cemadotin, administered every 21 days, after establishing that all eligibility criteria were satisfied. Intrapatient dose escalation was not permitted. Patients were withdrawn from the study if any of the eligibility requirements remained unacceptable 5 weeks after dosing. Treatment was also discontinued upon documenting tumor progression or a complete response.

Response criteria

Patients were evaluated for therapeutic response after completing every second cycle of therapy. Tumors were measured by radiologic methods and/or physical examination. Tumor burden was calculated as the sum of the products of the longest perpendicular diameters of all measurable lesions. Tumor measurements were repeated bimonthly until relapse. The duration of a response was measured from the date that the response was first recorded to the date of documented disease progression. Responses were defined as in the previous studies of cemadotin [14, 16].

Specimen collection and quantitation

Serial blood sampling to characterize the pharmacokinetics of cemadotin was performed during the first cycle of treatment. Blood was drawn from a peripheral venous catheter placed in the arm of the patient into Vacutainer Brand tubes with liquid EDTA (K_3) anticoagulant (Becton Dickinson, Franklin Lakes, N.J.). Patency of the catheter was maintained with the use of a heparin lock. Specimens (10 ml) were collected shortly before treatment, at 4, 6, 24, 48, 72, 96, and 120 h after starting the infusion, and 0.25, 0.5, 1, 3, 6, 8, 24, and 48 h following the end of the infusion. The whole blood was placed on ice until transferred into polypropylene cryovials. Urine was collected from 96 to 120 h after starting the infusion. The volume of the pooled 24-h urine collections was measured and, after thoroughly mixing, an aliquot (15–20 ml) was retained for storage in a borosilicate glass vial. The whole blood and urine specimens were stored at −20 °C or lower until thawed for analysis. The reading of a battery-powered digital timer/stopwatch was recorded when the infusion pump was started or stopped, when blood samples were collected, and at the beginning and end of the urine collection interval.

The concentration of cemadotin in whole blood and urine specimens was determined by investigators at BASF/Knoll AG (Ludwigshafen, Germany) using a radioimmunoassay (RIA), as previously described [14]. Briefly, the assay was based upon the competition of cemadotin and an iodine-125 labeled hydroxyphenyl derivative of cemadotin (LU 109864) for binding to polyclonal rabbit antibodies. Free and antibody-bound radiotracer were separated by immunoprecipitation using goat anti-rabbit IgG, and γ-radiation in the antibody-bound fraction was measured. Detector response was inversely proportional to the concentration of cemadotin in the sample, since binding of the radiotracer to the antibody was limited by the amount of bound cemadotin. Studies to establish the specificity of the assay revealed that the antibody recognized the amino-terminal region of the molecule and cross-reacted with carboxy-terminal derivatives of cemadotin with 100% efficiency. Furthermore, preclinical studies demonstrated that the C-terminal benzylamine functional group of cemadotin is subject to rapid enzymatic hydrolysis in human whole blood in vitro. The resulting desbenzylamine metabolite (LU 117319, Fig. 1) proved to be indistinguishable from the parent drug by the assay; therefore, the results obtained have been expressed as cemadotin RIA equivalents.

Blood samples were subject to several freeze-thaw cycles to completely lyse cells prior to analysis. Urine was assayed directly upon thawing without any pretreatment. Samples were batched and sent to a central laboratory for analysis. All study samples were assayed in duplicate together with a series of calibration standards prepared by spiking whole blood or urine with desbenzylaminocemadotin to provide a series of nine solutions with concentrations ranging from 10.0 to 360 n*M* in blood and from 1.14 to 20.0 n*M* in urine. Calibration curves were analyzed using a four-parameter logistic model. Values of the parameters describing the best fit curve were used to calculate the analyte concentration in the study samples. The limit of detection of the assay was 6.6 n*M* using 5 μl of whole blood and 0.74 n*M* for 50 μl of urine. Between-day accuracy of the assay for determining cemadotin equivalents in whole blood and urine was 95.3%–107.1%, with precision ≤15% throughout the entire concentration range of the standard curves.

Pharmacokinetic data analysis

Actual sample times relative to the beginning of the infusion were calculated and used for the pharmacokinetic analysis of the data. The apparent steady-state concentration of cemadotin RIA equivalents in blood (C_b^{ss}) was calculated as the geometric mean of the four measured values made from 48 to 120 h during the infusion of drug to each patient. Time courses of the cemadotin RIA equivalent concentration in blood for individual patients were analyzed by noncompartmental methods using the WinNonlin Professional version 1.5 software package (Scientific Consulting, Apex, N.C.) [21]. The combined amount of drug and intact N-terminal metabolites excreted in the urine during the final 24 h of the infusion, $A_e(96 \rightarrow 120\ h)$, was calculated as the product of the concentration measured by RIA and the total volume of the pooled urines. Renal clearance (CL_R) was estimated as

$$CL_R = A_e(96 \rightarrow 120\,h)/[AUC_b(96 \rightarrow 120\,h) \times BSA]$$

where $AUC_b(96 \rightarrow 120\ h)$ is the area under the blood concentration-time curve during the urine collection interval and BSA is the patient's body surface area in m^2 [22]. $AUC_b(96 \rightarrow 120\ h)$ was calculated from the equation for the area of a trapezoid using values of the blood concentrations measured at approximately 96 and 120 h with the actual beginning and ending times of the urine collection period to establish the time interval.

Values of the pharmacokinetic parameters and variables at each dose level are reported as the arithmetic mean ± SD of the individual patient data, except for the $t_{1/2,z}$ and MRT, which were calculated as harmonic means [23], and the geometric mean of apparent C_b^{ss} values are given. The SD for geometric and harmonic mean values was estimated by the jacknife technique [24].

The geometric mean of the cemadotin RIA equivalent blood concentrations and average of the actual sample times were calculated at each nominal time point from the individual patient data for groups that received the same dose. The model-independent equation for zero-order IV drug input and monoexponential disposition [22] was fit to the geometric mean blood concentration-time profiles by nonlinear least squares regression using WinNonlin, with $1/y_{obs}$weighting.

Pharmacodynamic assessments

Neutrophil count nadirs were based upon the observed minimum value following the first cycle of therapy with cemadotin. The maximum percent decrease was calculated as ((pretreatment count – nadir)/pretreatment count) ×100, where the pretreatment count was the determination made immediately prior to dosing. The maximum percent decrease was considered to be zero (i.e., unchanged) if all post-treatment determinations of the absolute neutrophil count were greater than or equal to the pretreatment value. Pearson's sample correlation coefficient (r) was calculated to assess the relationship between the relative neutrophil count nadirs and the total dose administered or the AUC_b of cemadotin.

Results

Patient characteristics

A total of 21 patients were enrolled into the study. However, one patient who developed hypercalcemia during the time between signing the consent form and initiating therapy 6 days later was excluded from further analysis. Thus, 20 patients were treated at five dose levels of cemadotin and evaluated for toxicity and response. Demographic characteristics for the entire cohort are summarized in Table 1. The study was representative of a heavily pretreated group of solid tumor patients similar to those entered into other phase I trials at our institutions.

Toxicity

All significant toxicities encountered in this phase I dose escalation trial of cemadotin are summarized in Tables 2 and 3. There were no treatment-related toxicities more severe than grade 2 following the first infusion of drug to cohorts of three or four patients at each of the first four dose levels of 2.5, 5.0, 8.25, and 12.5 mg/m^2. The DLT of cemadotin given as a 120-h CIV infusion proved to be neutropenia, which occurred during the first cycle of therapy in all patients treated at the fifth dose level of 17.5 mg/m^2 (Table 2). Despite the life-threatening degree of neutropenia in the patients who received 17.5 mg/m^2 cemadotin, as indicated by an absolute neutrophil count nadir below 500/μl, its duration was relatively short, persisting for less than 5 days. Although the protocol defined grade 4 hematologic DLT as >5 days, the severity of the neutropenia prompted us to declare the 17.5 mg/m^2 dose unacceptably toxic for any potential phase 2 study. Two of the four patients were retreated (a third received further treatment for 3/5 days before withdrawing due to progressive disease) at the

Table 1 Patient characteristics (n = 20)

Characteristic	Value
Age (yr)	
Median	58
Range	20–75
Sex (n)	
Male	15
Female	5
Performance status (n)	
0	7
1	11
2	2
Primary tumor site (n)	
Breast	1
Colorectal	9
Esophageal	1
Lung	2
Prostate	1
Renal	1
Sarcoma	3
Unknown	2
Prior chemotherapy regimens (n)	
1	4
2	7
3	3
≥4 (range)	6 (4–7)
Prior radiation therapy (n)	9
Total no. of courses for all therapies:	40
Median	2
Range	1–4

n, number of patients

Table 2 Classification, graded severity, and frequency of hematologic toxicities

Total dose (mg/m^2)	No. of courses	No. of occurrences of toxicity (grade 2/grade 3/grade 4):			
		Anemia	Thromocytopenia	Leukopenia	Neutropenia
Course 1					
2.5	3	1/0/0	0/0/0	0/0/0	0/0/0
5.0	3	0/0/0	0/0/0	0/0/0	0/0/0
8.25	4	0/0/0	0/0/0	1/0/0	0/0/0
12.5	6	1/0/0	0/0/0	1/0/0	1/0/0
17.5	4	2/0/0	1/0/0	0/3/1	0/0/4
All courses					
2.5	5	1/0/0	0/0/0	0/0/0	0/0/0
5.0	6	0/0/0	0/0/0	0/0/0	0/0/0
8.25	8	0/0/0	0/0/0	1/0/0	0/0/0
12.5	13	1/0/0	0/0/0	1/0/0	1/0/1
17.5	7	2/0/0	1/0/0	0/3/1	0/0/4

Table 3 Classification, graded severity, and frequency of non-hematologic toxicities

Total dose (mg/m^2)	No. of courses	No. of occurrences of toxicity (grade 2/grade 3 + 4):					
		Nausea	Diarrhea	Cardiac	Bilirubin	Hepatic[a]	Fatigue
Course 1							
2.5	3	0/0	0/0	0/0	0/0	0/0	0/0
5.0	3	0/0	0/0	0/0	0/0	0/0	0/0
8.25	4	0/0	0/0	0/0	0/0	0/0	1/0
12.5	6	2/1	0/0	0/0	0/1	0/0	2/0
17.5	4	1/0	1/0	0/0	0/1	0/0	2/0
All courses							
2.5	5	0/1	0/0	0/0	0/2	1/2	0/0
5.0	6	0/0	0/0	0/0	0/0	0/1	0/0
8.25	8	0/0	0/0	0/0	0/0	0/0	0/0
12.5	13	2/1	0/0	0/0	0/3	0/0	2/0
17.5	7	1/0	0/0	0/0	0/1	0/0	2/0

[a] Elevated serum transaminase activities

17.5 mg/m^2 dose with slightly less hematologic toxicity on the second course. Subsequently, an additional three patients were evaluated at the fourth dose level of 12.5 mg/m^2. There was hematologic toxicity greater than grade 2 in a single patient (grade 4 neutropenia) and a single dose-limiting nonhematologic toxicity in the six patients during the first course of treatment at this dose level. Accordingly, 12.5 mg/m^2 was established as the MTD of the drug for this schedule. There were no incidences of fever or evidence of sepsis. Moderate (i.e., grade 2) anemia was observed in 4/20 patients and a single patient treated with 17.5 mg/m^2 cemadotin experienced moderate thrombocytopenia.

Table 3 lists the nonhematologic toxicities encountered during the course of the study. Frequent blood pressure monitoring and acquisition of electrocardiograms failed to reveal any evidence of hypertension or cardiac ischemia, despite the male predominance of the patients studied and their median age of 58 years. Hepatoxicity manifest as hyperbilirubinemia was observed in three of six patients treated at the MTD of 12.5 mg/m^2. In each case, the bilirubin elevation appeared to be the result of progressing liver metastases from primary colorectal cancer. Two other patients with progressive metastatic cancer to the liver developed grade 3 hyperbilirubinemia and other hepatic chemistry abnormalities at the starting dose (2.5 mg/m^2). Moderate fatigue occurred in four of ten patients treated at or above the MTD. Nausea and vomiting were infrequent and most patients did not require antiemetic medications, which was not used prior to the first cycle of cemadotin unless specifically requested by the patient. The single patient who did experience severe nausea and vomiting had a small bowel obstruction due to progressive metastatic colorectal cancer. No other gastrointestinal toxicities were evident except for moderate (grade 2) diarrhea in a single patient. Two patients, who were treated at dose levels 1 (2.5 mg/m^2) and 4 (12.5 mg/m^2), developed severe dyspnea upon exertion. This was considered to be associated with progressive metastatic cancer rather than to be a drug-related event.

Responses

There were no objective responses to cemadotin among the 20 patients who were evaluated for response. Apparent disease stabilization was noted in two patients evaluated after receiving the initial two cycles of therapy, with progressive disease demonstrated following an additional two cycles of the drug.

Pharmacokinetics

Pharmacokinetic studies were performed in groups of three patients at each dose level, with the exception of the third dose level of 8.25 mg/m^2, for which data are available from only two patients. The cohort included nine males and five females with a mean ± SD age of 53 ± 15 years (range 20–75 years). Geometric mean blood concentration-time profiles of cemadotin RIA equivalents determined at each of the five dose levels evaluated are shown in Fig. 2. Blood levels increased gradually during the first 2 days of the infusion and became essentially constant from 48 h after dosing until the infusion was terminated. The apparent C_b^{ss} of cemadotin RIA equivalents, calculated from the geometric mean of the observed concentrations at 48–120 h in individual patients, increased from a mean value of 62 ± 17 nM ($n = 3$) at the starting dose of 2.50 mg/m^2 to 417 ± 100 nM ($n = 3$) in the cohort treated with 17.5 mg/m^2, the highest dose administered. Mean values of the pharmacokinetic variables estimated by noncompartmental analysis of the individual patient data are presented in Table 4. The extrapolated region of the time course following the last measurable concentration, being less than 2.6% of the AUC_b on average (range 0.4%–9.6%), had a relatively insignificant effect upon the estimated values.

Although the data are based upon the combined concentrations of parent drug and at least one major metabolite [11], which are indistinguishable by the analytical method employed, the pharmacokinetics of cemadotin RIA equivalents nevertheless appears to be linear. This is inferred by the proportionate increases in the AUC_b ($r = 0.997$) and the apparent C_b^{ss} ($r = 0.998$) with escalation of total administered dose. The grand mean total blood clearance (CL_b) was 0.515 ± 0.092 l/h/m^2 in the 14 patients evaluated. The magnitude of the apparent terminal phase half-life ($t_{1/2,z}$) and mean residence time MRT were comparable, with mean values ($n = 14$) of 13.2 ± 4.3 h and 18.0 ± 3.2 h, respectively. The apparent volume of distribution at steady state (V_{ss}) was relatively small, with a grand mean of 9.9 ± 3.3 l/

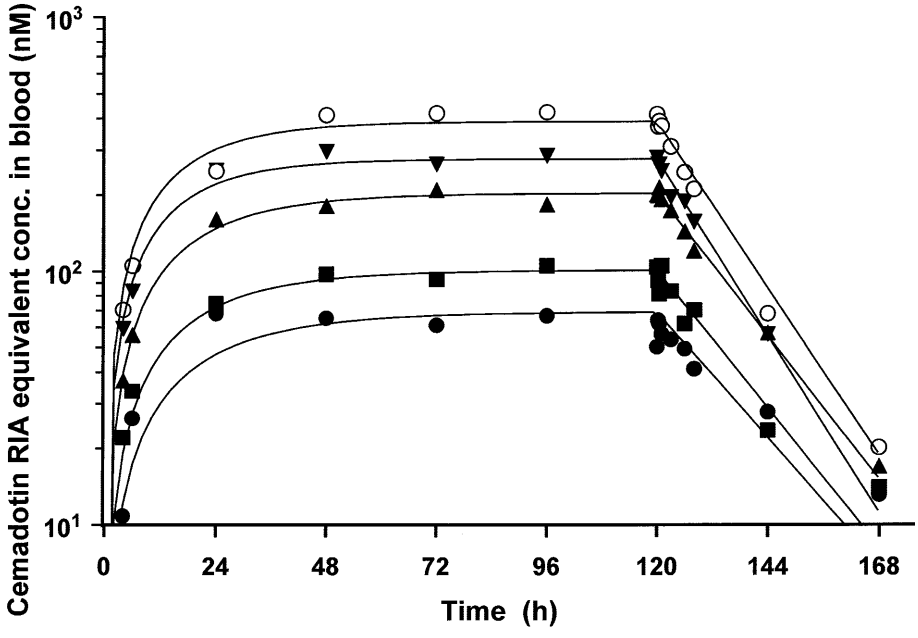

Fig. 2 Geometric mean blood concentration-time profiles of cemadotin RIA equivalents in groups of cancer patients treated with 2.5 mg/m^2 (●), 5.0 mg/m^2 (■), 8.25 mg/m^2 (▲), 12.5 mg/m^2 (▼), and 17.5 mg/m^2 (○) doses of the drug given by 120-h CIV infusion. The observed blood concentrations (*points*) are shown together with best-fit curves (—) determined by nonlinear regression analysis

Table 4 Summary of mean pharmacokinetic parameters estimated by noncompartmental analysis of the time courses of cemadotin RIA equivalents in whole blood

Total dose (mg/m^2)	No. of patients	C_b^{ss} (nM)	AUC_b (μM · h)	$t_{1/2}$ (h)	CL_b (l/h/m^2)	MRT (h)	V_{ss} (l/m^2)	CL_R (l/h/m^2)
2.50	3	62 (17)[a]	9.4 (1.4)	20.6 (4.3)	0.399 (0.065)	20.3 (7.2)	9.5 (5.7)	0.328 b
5.00	3	100 (3)	12.8 (0.8)	17.0 (2.9)	0.580 (0.038)	21.5 (4.2)	12.8 (2.8)	0.124 (0.032)
8.25	2	193 (30)	24.5 (3.2)	13.5 (5.1)	0.499 (0.061)	18.9 (5.1)	9.5 (1.0)	0.203 (0.080)
12.50	3	282 (7)	34.2 (2.4)	9.2 (2.1)	0.542 (0.040)	14.2 (2.1)	7.8 (0.9)	0.277 (0.095)
17.50	3	417 (100)	48.8 (10.5)	11.3 (1.7)	0.549 (0.128)	17.3 (4.2)	9.9 (2.9)	0.334 (0.123)
Mean (SD)				13.2 (4.3)	0.515 (0.092)	18.0 (3.2)	9.9 (3.3)	0.312 (0.178)
CV (%)				32.7	17.9	17.7	33.3	57.0

[a] Numbers in parentheses, standard deviation
[b] Based upon data from a single patient

m^2 ($n = 14$), being approximately 27% of total body weight. This finding would suggest that the RIA-detectable species originating from the drug are largely confined to systemic circulation and extracellular fluid, consistent with the highly polar character of the positively charged drug and desbenzylamino metabolite, which exists as a zwitterion under physiologic conditions. The pharmacokinetic parameters exhibited a moderate degree of interpatient variability, as the CV ranged from 17.7 to 33.0 for the mean values for the entire cohort of patients. Urinary excretion was the predominant route of elimination for unchanged drug and RIA-detectable metabolites, accounting for 60.6% of the administered dose on average.

While the individual patient blood concentration-time profiles were generally not amenable to nonlinear regression analysis, a marked improvement in the data was afforded by calculating the geometric mean of the observed RIA equivalent cemadotin concentration at each time point for groups of patients who received the same dose. As depicted in Fig. 2, the geometric mean profiles were very well described by the equation for CIV infusion of drug with first-order monoexponential loss of RIA-detectable species from blood following the end of the infusion. The description of the data was not improved by fitting an equation with biexponential disposition. However, the possibility remains that timing of sample acquisition during the immediate postinfusion period and/or a high degree of variability in the observed blood concentrations could have precluded elucidation of a more rapid initial disposition phase. Values of the pharmacokinetic variables afforded by nonlinear regression analysis of the pooled patient data (not shown) were in excellent agreement with mean values provided by noncompartmental analysis of the individual patient data.

Pharmacodynamics

Hematologic toxicity manifested predominantly as neutropenia was observed in all but 2 of the 20 patients entered into the study. Neutrophil count nadirs were achieved 2–3 weeks after initiating the infusion of cemadotin, and the cell count returned to baseline levels in all patients without evidence of any cumulative effects. As demonstrated in Fig. 3, the relative degree of neutropenia during the first cycle of therapy, expressed as the maximum percent reduction from pretreatment values, was strongly correlated with the dose of cemadotin ($r = 0.847$) as well as the AUC_b ($r = 0.810$). Although the maximum percent decrease in neutrophil counts approached 100% in patients exhibiting the greatest AUC_b values, an insufficient number of patients were studied at the higher dose levels to discern a distinct plateau in the hematologic response-AUC_b profile. Thus, meaningful parameter estimates were not obtained during efforts to fit the typical sigmoidal or hyperbolic pharmacodynamic models to the experimental data.

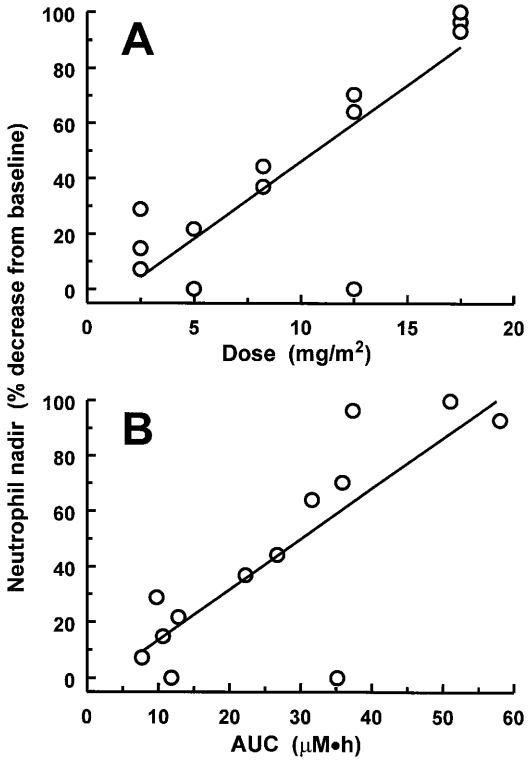

Fig. 3 Relationship between the neutrophil count nadir, expressed as the percent decrease from the pretreatment value, during the first cycle of therapy and **A** the total dose of cemadotin administered, and **B** the AUC_b of cemadotin RIA equivalents in whole blood. The *points* (○) represent the observed values in individual patients. *Solid lines* were generated from linear regression analysis of the experimental data. Values of Pearson's sample correlation coefficient were 0.847 and 0.810 for the relationship with dose and AUC_b, respectively

Discussion

Although natural products targeting the dynamics of microtubule assembly and depolymerization have been used clinically as anticancer agents for decades, the encouraging efficacy achieved with these compounds, coupled with the more recent success of the taxanes, engenders continued interest in the development of new antimitotic drugs. The dolastatins are a series of small peptides isolated from a marine organism with broad-spectrum cytotoxic activity that appears to result from their interactions with tubulin and the microtubule network [2, 3]. Dolastatin 10 was the first member of this class to be selected for clinical evaluation on the basis of its cytotoxic potency, antitumor activity, and supply. Interest in these compounds was heightened by the economical synthesis of structurally related peptides with better pharmaceutical characteristics than the natural products, but with comparable biologic activity [8, 9]. Cemadotin represents the first, and remains the only, synthetic dolastatin analogue that has been clinically evaluated as a chemotherapeutic agent.

Cemadotin was introduced into phase I clinical trials as a 5-min IV injection repeated at 3-week intervals [11]. Preclinical toxicology studies identified highly proliferative organs such as bone marrow, the thymus, and intestinal epithelium as being the principal targets of drug toxicity. However, only one patient who was treated with the maximum administered dose of 20 mg/m^2 cemadotin experienced significant myelotoxicity. Reversible hypertension and myocardial infarction, occurring at the 10 and 20 mg/m^2 dose levels, proved to be the DLTs. Other nonhematologic toxicities, which included nausea and vomiting, mucositis, fever, and pain at the tumor site, were generally mild to moderate in severity. The nature of the DLTs was unanticipated [12]. Cemadotin is a relatively weak agonist of the angiotensin II type 1 receptor, producing a dose-dependent increase in diastolic blood pressure in rats and dogs that can be completely normalized by angiotensin-converting enzyme inhibitors [11].

Several additional phase I studies of cemadotin were subsequently initiated to determine whether the risk of cardiovascular toxicity could be minimized by modifying the dosing schedule and duration of infusion [12, 13, 14, 15]. These studies have clearly demonstrated that the DLTs of this drug are highly schedule dependent. Neutropenia became a significant toxicity upon increasing the duration of infusion from 5 min to 24 h for the once every 3 weeks administration schedule, although cardiovascular toxicity remained dose limiting [12]. Decreasing the dosing interval from 3 weeks to 1 week further diminished the cardiovascular effects of the 24-h infusion and neutropenia became the DLT [14], whereas the once weekly ×4 schedule remained hypertensive for the 5-min IV injection [16]. Neutropenia was also the DLT of cemadotin when given as a single bolus IV injection for five consecutive days [16]. However, the frequency of systolic and/or diastolic hypertension was 30% for all courses of therapy at or below the MTD of 12.5 mg/m^2 (2.5 mg/m^2 daily). In addition, three patients who received total weekly doses of 12.5 or 15.0 mg/m^2 developed grade 3–4 peripheral edema, and a single patient showed evidence of myocardial ischemia.

In the present study, 20 heavily pretreated patients with advanced cancer received a total of 40 courses of cemadotin, administered as a 120-h CIV infusion, over five dose levels ranging from 2.5 to 17.5 mg/m^2. This schedule was extremely well tolerated from the perspective of nonhematologic toxicity, with absolutely no evidence of cardiovascular toxicity. Nausea was generally mild and satisfactorily controlled with phenothiazine antiemetics. The only grade 3 nonhematologic toxicity observed was elevated serum bilirubin in four patients. In each case, this was attributed to progression of known metastatic cancer to the liver. Neutropenia was the principal DLT and 12.5 mg/m^2 was established as the MTD for this schedule. It was observed in all but two of the 20 patients in the study and became dose limiting at 17.5 mg/m^2. The effect was not cumulative and resolved within the 21-day period between cycles such that no patient required a treatment delay for myelosuppression. The relative degree of neutropenia was linearly related to both the dose and AUC_b.

Two phase I clinical trials of dolastatin 10 administered as a bolus IV injection repeated at an interval of 21 days have been undertaken [6, 25]. Unlike cemadotin, only one episode of mild (grade 1) blood pressure elevation occurred following the administration of 94 cycles of dolastatin 10 to 30 patients [6]. The MTD of dolastatin 10 was found to be 0.400 mg/m^2 for patients previously treated with ≤ two chemotherapy regimens and 0.325 mg/m^2 for more heavily pretreated patients. Granulocytopenia proved to be the DLT. The other frequently encountered toxicities included local irritation and phlebitis at the injection site, and mild peripheral sensory neuropathies.

Values of the pharmacokinetic variables determined in cancer patients treated with the 120-h CIV infusion of cemadotin were very similar to the findings of prior phase I studies of the drug given by 5-min injection or 24-h infusion. Among the 14 patients from whom pharmacokinetic data were obtained, blood levels decayed with a $t_{1/2,z}$ of 13.2 ± 4.3 h after the 120-h infusion had ended, the CL_b was 0.52 ± 0.09 l/h/m^2, and the V_{ss} was 9.9 ± 3.3 l/m^2 (mean ± SD). In comparison, the range of values for these parameters reported in other phase I trials of the drug were 9.4–12.3 h for $t_{1/2,z}$, 0.5–0.8 l/h/m^2 for CL_b, and 7.6–9.6 l/m^2 for V_{ss} [14, 15, 16]. Thus, it would appear that the pharmacokinetics of the drug is linear across a broad range of doses and independent of the duration of infusion.

In each of the phase I studies of cemadotin, including the present investigation, the pharmacokinetic data were based upon measurements in whole blood using the same RIA. The polyclonal antibody employed in the assay binds to the N-terminal region of the molecule and, therefore, does not distinguish metabolites with C-terminal structural alterations from the parent drug [14]. It is important to realize, therefore, that the assay was not specific for cemadotin. Preclinical pharmacology studies showed that the C-terminal benzylamide functional group of the drug was rapidly cleaved by cytosolic endopeptidases in blood cells [14]. The resulting desbenzylamino metabolite, which was completely indistinguishable from cemadotin by the RIA, appeared to be the predominant drug-related compound present in the whole blood of animals following the IV administration of cemadotin. Furthermore, the concentration of RIA-detectable species in plasma rapidly decayed below measurable levels, while relatively high concentrations persisted in whole blood. Evidently, the parent drug proficiently distributes into blood cells upon presentation to the bloodstream, whereupon it is rapidly hydrolyzed to a highly polar metabolite that accumulates within the cells, from which the metabolite is slowly released and rapidly eliminated from the body, primarily by urinary excretion. *N*-Desbenzylamino-cemadotin retains the tubulin interactive properties of the parent drug in cell-free systems; however, it has little antiproliferative activity owing to limited cellular uptake [15].

On the basis of these findings, cemadotin has been described as being a prodrug [14], albeit a characterization that is not consistent with the known pharmacokinetic behavior of the compound. By definition, a prodrug is an inactive bioreversible derivative designed to solve a problem in delivering a drug to its intended site of action whereupon it efficiently converts back to the parent drug molecule [26]. In contrast, it is likely that the large majority of administered cemadotin is metabolized and retained within blood cells prior to distribution to solid tumors and other slowly perfused tissues. This premise is consistent with the relatively low V_{ss} observed in this and other phase I studies, which is approximately 27% of total body weight, thereby suggesting that the RIA-detectable species originating from the drug are largely confined to the bloodstream and rapidly perfused tissues. Accordingly, it is plausible that only a small fraction of the administered dose represents therapeutically available drug. The principal toxicities of the drug, neutropenia and cardiovascular effects, are associated with rapidly perfused tissues that are presumably exposed to the intact drug present in plasma during the infusion and immediately thereafter.

Blood levels of the RIA-detectable species achieved steady state within 48 h after initiating the 120-h infusion of cemadotin. The mean C_b^{ss} in the three patients treated at the 12.5 mg/m^2 MTD, 282 ± 7 n*M*, was much greater than the in vitro 50% inhibitory concentration of cemadotin against human tumor cell lines, which ranged from 0.2 to 1 n*M* for 72 h of continuous exposure to the drug [9, 10]. The pharmacokinetic behavior of the drug cannot be compared with that of dolastatin 10 in cancer patients because the concentration of dolastatin 10 was measured in plasma, rather than whole blood, using either a biologic assay [6] or a more specific method based upon high performance liquid chromatography with mass spectrometric detection [25, 27].

The 12.5 mg/m^2 MTD of cemadotin for the 120-h CIV schedule was the same as the MTD reported for the 5-min IV injection daily times 5 schedule in a cohort of cancer patients with similar characteristics [16]. In comparison, the therapeutically optimal dosing regimen in mice was a total dose of 90 mg/kg (270 mg/m^2) given as three bolus IV injections once every other day [13]. Thus, tumor-bearing mice tolerated a substantially greater dose intensity of cemadotin than humans. Moreover, the therapeutic effects were achieved at doses that were more than 20 times higher than permitted by the two most promising dosing regimens of the drug in cancer patients. There were no patients with an objective therapeutic response to cemadotin delivered as a 120-h CIV infusion and only two patients showed indications of disease stabilization. This is not unusual for a phase I trial involving heavily pretreated patients with varying tumor types, for which fewer than one-half of the patients received the MTD or higher. However, no objective responses were observed in at least 101 patients entered into the other five clinical trails [14, 15, 16, 17, 18], with only two patients showing evidence of disease stabilization [14]. Similarly, there were no objective responses among 30 patients treated with 107 cycles of dolastatin 10 [6].

In summary, this study has shown that cemadotin produces noncumulative dose-limiting neutropenia when administered as a 5-day CIV infusion. The MTD of the drug for this schedule was established as being 12.5 mg/m^2. Nonhematologic toxicities were generally mild and there was no evidence of any cardiovascular toxicity, which had been prevalent in all prior phase I studies of cemadotin when administered as a 5-min IV injection or 24-h infusion according to various schedules. When considered together with observations from other clinical trials of cemadotin, this finding confirms that cardiovascular toxicity is associated with the magnitude of the peak blood levels of the parent drug or its metabolites, whereas myelotoxicity is related to the duration of time that blood levels exceed a threshold concentration [16]. This study has confirmed that the doses of cemadotin tolerated by humans are markedly lower than those required for antitumor effects in preclinical animal models. A substantial fraction of the administered dose of cemadotin is rapidly sequestered by blood cells and converted to a therapeutically unavailable metabolite. In consideration of the pharmacokinetic behavior, together with the lack of antitumor activity in a large cohort of patients, further clinical evaluation of this particular dolastatin analogue may not be warranted.

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