Eur J Cancer Clin Oncol, Vol. 24, No. 11, pp. 1699–1706, 1988.
Printed in Great Britain

0277–5379/88$3.00 + 0.00

Phase I Clinical and Pharmacokinetic Investigation of Didemnin B, a Cyclic Depsipeptide*

F. ANDREW DORR, JOHN G. KUHN, JERRY PHILLIPS and DANIEL D. VON HOFF

University of Texas Health Service Center, Department of Medicine, Division of Oncology, San Antonio, TX 78284, U.S.A. and Cancer Therapy and Research Center, San Antonio, TX 78228, U.S.A.

Abstract—*Didemnin B (NSC-325319), a cyclic depsipeptide isolated from a marine tunicate, has been evaluated in a Phase I trial. The drug was administered in a single intravenous infusion in* 150 cm^3 *of normal saline every* 30 *min given every* 28 *days. Forty-three patients received* 80 *courses of the drug at doses ranging from* 0.14 *to* 4.51 mg/m^2. *The dose-limiting toxicity was nausea and vomiting which began during or shortly after the infusion and was of variable duration. This toxicity was somewhat ameliorated by pretreatment with an aggressive antiemetic regimen. Mild hepatic toxicity also occurred with mild elevations of transaminases and bilirubin. One patient experienced an allergic reaction during his second infusion, characterized by chills, diaphoresis, flushing and hypotension. No objective anti-tumor response was seen during this trial. The recommended dose for Phase II studies on a single-dose schedule is* 2.67 mg/m^2 *without prophylactic antiemetics and* 3.47 mg/m^2 *if an antiemetic regimen is used. Preliminary pharmacokinetics suggest that didemnin B is sequestered or rapidly converted to a metabolite not identified by the antibody used in the radioimmunoassay. Further evaluation will be performed during Phase II studies.*

INTRODUCTION

DIDEMNINS represent a new class of depsipeptides which were isolated in 1978 by Rinehart *et al.* from a species of the *Tridemnum* genus of the Didemnidae family. The initial antiviral and cytotoxic activities of this class of compounds were identified during shipboard testing [1, 2]. Subsequent chemical studies isolated three separate compounds, identified as didemnins A, B and C. Each compound contained the same cyclic depsipeptide with a distinct substituent attached at the *N*-methylleucine amino acid. Didemnin B, with lactylproline attached (Fig. 1), has been found to be the most potent of the didemnins with *in vitro* activity against both RNA and DNA viruses as well as L1210 leukemia cells [1, 2]. In the NCI screening program, didemnin B demonstrated *in vivo* antitumor activity against i.p. implanted B16 melanoma, M5076 sarcoma and P388 leukemia [3].

Preclinical toxicologic evaluation of didemnin B conducted in CD_2F_1 mice, Fischer 344 rats and beagle dogs identified the lymphatics, gastro-intestinal tract, liver and kidney as the major target organs. In dogs, dose-related gastrointestinal bleeding and increased prothrombin times were the most severe toxicities [3]. Subsequent studies revealed that didemnin B produces an inhibition of hepatic parenchymal cell protein synthesis which results in reduced production of clotting factors VII, VIII:C, IX, X and XI [4].

The initial studies on the mechanism of action of this drug have shown it to be a potent protein synthesis inhibitor and to a lesser extent an inhibitor of DNA and RNA synthesis [5]. Flow cytometry results have shown that low doses of didemnin B inhibit progression of B16 melanoma from G_1 to S phase without significant inhibition of progression from S phase to G_2 + M to G_1 [6]. In addition, didemnin B was more lethal to exponentially growing B16 cells than to plateau phase cells. This drug does not, however, appear to be cell-cycle phase specific since at higher doses it is lethal to and arrests cells in each phase of the cell cycle [6].

Didemnin B has been studied in the human tumor cloning assay [7, 8]. Results of these studies indicate that didemnin B could prove to be an active agent in clinical trials if concentration–time products in the range of 0.1–1.0 μg-h/ml are achievable [7].

Accepted 13 June 1988.
Address correspondence to: Andrew Dorr, M.D., Executive Plaza N, Rm 741F, Bethesda, MD 20892, U.S.A.
*This study was supported by NCI Contract No-1 CM-57737, NIH Grant RR-01346, PHS National Research Service Award CA-09434 and the VA Research Service.

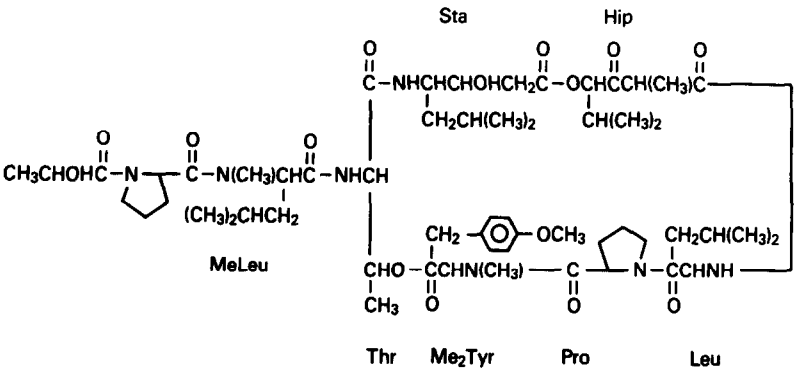

Fig. 1. Structure of didemnin B. Sta, Statine; Hip, α-(α-hydroxy-isovaleryl) propionic acid.

MATERIALS AND METHODS

Selection of patients

All patients who entered this trial had histologic confirmation of cancer. Only patients who had an estimated survival of >12 weeks and performance status less than or equal to three were eligible. All patients had measurable and/or evaluable disease at the time of entry. Nineteen of the 43 patients had received both prior radiotherapy and chemotherapy; 12, prior chemotherapy only; eight, prior radiotherapy only; one, radiotherapy and gamma interferon; and three had received no prior therapy.

Prior to study entry all patients had adequate blood cell counts (WBC, $\geq 3000/mm^3$; granulocytes, $\geq 1500/mm^3$; platelets, $>100{,}000/mm^3$). Other criteria included: (a) bilirubin, <2.5 mg/dl; (b) serum glutamic–oxaloacetic acid transaminase, <2.0-fold greater than normal; (c) creatinine, ≤2.0 mg/dl; (d) normal prothrombin and partial thromboplastin times.

Prior to study entry, informed consent was obtained from each patient in accordance wth federal and institutional policies.

Treatment plan

The formulation of didemnin B for clinical use was supplied by the National Cancer Institute's Division of Cancer Treatment (NCI-DCT) as a sterile 1 ml ampule containing 1 ml of drug; ethanol 5% v/v; Cremophor EL 5% v/v and normal saline. The single use vial contained no antibacterial preservative and drug from each vial was used within 8 h of being opened.

The appropriate dose of didemnin B was removed from the vial(s) and was diluted in 150 cm^3 of normal saline. The resulting clear solution was infused over 30 min through a newly started, freely flowing i.v. line. The schedule of drug administration was a single dose given every 28 days. For pharmacokinetic studies, sampling times were measured from the time of completion of the drug infusion.

Because of the abnormalities of clotting times in the dog toxicity studies, the starting dose for this trial was reduced to one-third of one-tenth the mouse 10% lethal dose which was 4.20 mg/m^2 on the single dose schedule. Therefore, the starting dose in this study was 0.14 mg/m^2. Three patients were entered at each dose level, using the modified Fibonacci schema for dose escalation. Additional patients were added at several dose levels to further define toxicity. Dose escalation was implemented in only three patients at lower dose levels. Otherwise dose escalation for individual patients was not performed in order to determine whether cumulative toxicity would occur with sequential courses every 28 days.

Study parameters

Prior to and during infusion of the drug and for 48 h following the end of infusion, patients had frequent monitoring of their electrocardiogram and vital signs. A complete blood count, including differential and platelets, chemistry profile, prothrombin and partial thromboplastin times, fibrinogen and fibrin split products, uric acid, creatinine and urinalysis were obtained prior to treatment and weekly for 4 weeks after each course. Electrocardiograms and chest radiographs were obtained prior to each course of treatment and at the end of treatment. Ophthalmologic examinations were also performed prior to each course and at the end of treatment because of occasional ocular abnormalities in the dog studies. Other laboratory and radiological examinations pertinent to tumor response were performed prior to each course. Patients were followed closely for signs of toxicity or other biological effects. Standard response criteria were used to evaluate the antitumor effect of the drug.

Termination of study

Individual patients were removed from the study if objective tumor progression occurred following one or more courses of didemnin B. The Phase I

study was terminated when the maximally tolerated dose was established and all patients had either experienced tumor progression while on treatment, refused further therapy or experienced unacceptable toxicity.

Blood sampling

Heparinized blood samples were collected through an indwelling 21 gauge heparin lock established in the opposite arm of the drug infusion. At each sampling time, 1 ml of whole blood was withdrawn and discarded to assure that the heparin solution used to maintain catheter patency did not dilute the sample. Thirteen serial 9 ml blood samples were drawn at the following times: predose, 5, 10, 20, 40, 60 mins; 2, 4 6, 8, 12, 24 and 48 h post infusion. Samples were immediately centrifuged by cold centrifugation (4°C) at 2500 ***g*** for 12 min. The plasma was then separated into two aliquots, flash frozen and stored at −70°C until analysis.

Quantitative analysis of drug

Didemnin B was measured using a radioimmunoassay after efforts to develop a B16 melanoma bioassay were unsuccessful and a high-performance liquid chromatography (HPLC) assay quantitative to 50 mg/ml of didemnin B was not sufficiently sensitive. As the assay methodology has not been previously published it is presented here in detail.

Conjugation of didemnin A to protein was achieved relatively easily since the A form of the drug possesses an N-terminal proline residue, while didemnin B has an N-terminal *N*-lactyl proline. Didemnin A was provided by Dr. Kenneth L. Rinehart, Jr., School of Chemical Sciences, University of Illinois, Urbana, Illinois. Didemnin A (4.8 μmol) and bovine serum albumin (0.145 μmol) were dissolved in 1 ml of a 5:5:5:1 mixture of 1 M $NaHCO_3$:ethanol:water:dimethylformamide. *N*-Ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide dihydrochloride was added in 20 mg increments over a 5 h reaction period at 25°C. Protein was precipitated with acetone, centrifuged and washed twice with acetone. The final pellet was dried under a stream of nitrogen, yielding 12 mg of white powder. It was estimated from HPLC analysis of unconjugated didemnin A that the final product contained approx. 20 mol didemnin A per mol bovine serum albumin (BSA).

For immunization, didemnin A–BSA conjugate was suspended in 0.9% aqueous sodium chloride by sonication. This solution was mixed with Ribi adjuvant (Ribi ImmunoChem Research, Inc., Hamilton, MT) to yield an emulsion with a final concentration of conjugate of 1 mg/ml. Young female New Zealand white rabbits were each injected subcutaneously with 0.25 ml emulsion over each back leg and shoulder. Injections were performed at three week intervals until suitable antibodies titers were reached.

[^{3}H]-Acetyldidemnin B was prepared by dissolving didemnin B (0.83 μmol) in 1 ml of dry pyridine containing 2.1 μmol dimethylaminopyridine and 25 mCi [^{3}H]acetic anhydride (sp. act. 6.27 Ci.mmol; Amersham Corp., Arlington Heights, IL). The reaction was for 4 h at 70°C. The solution was then evaporated to dryness under vacuum and redissolved in a small volume of absolute ethanol. All of this solution was spotted on a silica thin layer chromatography (TLC) plate containing a fluorescent indicator. The plate was developed with acetonitrile:methylene chloride (1:1). In addition to a small amount of unreacted didemnin B (R_f = 0.65), the plate contained only one additional spot, which corresponded to acetylated didemnin B (R_f = 0.85) as judged by TLC of a parallel reaction employing unlabeled acetic anhydride. Silica containing the acetylated didemnin B was scraped from the plate and the modified compound was eluted with absolute ethanol. After quantitation and liquid scintillation counting, the specific activity of the [^{3}H]-acetyldidemnin B was determined to be approx. 13 Ci/mmol.

Throughout the radioimmunoassay the buffer diluent used was phosphate-buffered saline (pH 7.2) containing 0.1% (v/v) gelatin. The protocol used is shown in Table 3. All points were set up in duplicate. Didemnin B standards were prepared from serial dilutions of a stock solution of the compound (1 mg/ml in ethanol) that was maintained at −20°C. The dilutions were made in human plasma. The antibody was kept at −20°C until use. The amount to be used for the assay was determined by antibody dilution curves to be that dilution which bound 40–50% of the added [^{3}H]acetyldidemnin B. For the studies reported here, the antibody was diluted 1:60, resulting in a final dilution of 1:300 in the reaction mixture. [^{3}H]Acetyldidemnin B was stored as a concentrated stock solution in ethanol at −20°C and diluted in fresh buffer for each assay. The diluted working solution provided 0.5 pmol labeled compound (approximately 14–15,000 dpm) in each assay tube. Dextran-coated charcoal was prepared by stirring 40 g/l activated charcoal and 6 g/l Dextran T-70 in phosphate-buffered saline (PBS) at 4°C overnight. The suspension was then centrifuged, the supernatant decanted to remove fine charcoal particles and the coated charcoal resuspended in the same volume of PBS used originally and stored at 4°C.

To perform the assay, reagents were added, as indicated in Table 3, to 1.5 ml polypropylene microcentrifuge tubes. The contents were mixed and incubated at 37°C for 1 h. Tubes were then placed in a 4°C bath for 10 min. Dextran-coated charcoal

was resuspended and added to each tube which was then vortexed briefly. Assay tubes were incubated at 4°C for 10 min and then mixed again. Incubation was continued for an additional 10 min, at which time the tubes were mixed once more. After a final 10-min incubation at 4°C, tubes were centrifuged for 2 min in an Eppendorf microfuge. Supernatant (0.8 ml) was removed from each tube and placed in a liquid scintillation vial. After the addition of 10 ml ACS II cocktail (Amersham Corp.), samples were counted on a Beckman LS 9800 liquid scintillation counter.

For all samples, the mean dpm for each set of duplicates after subtraction of the mean non-specific binding (NSB) dpm was expressed as a percentage of the zero-binding dpm. In this assay, NSB was generally about 5% of the total dpm. Calibration curves were prepared daily and all samples were assayed in duplicates and replicated. Linearity of the standard curve was 0.9975 over the range of 10 pg to 10 ng with an intraassay and interassay variation of 5–10% and 10–15%, respectively (Fig. 2). Didemnin A and didemnin B competed equally with one another for binding to the antibody.

RESULTS

Over an 18-month period 43 patients were entered on study. Patient characteristics and tumor types are shown in Tables 1 and 2, respectively. Eighty courses of the drug were given with doses ranging from 0.14 to 4.51 mg/m^2. One patient

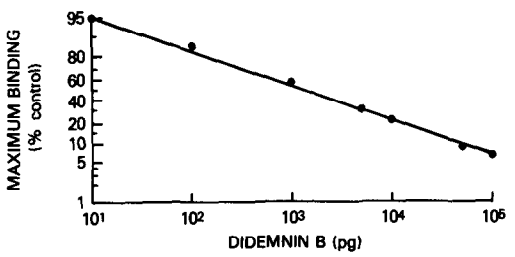

Fig. 2. Standard curve for didemnin B radioimmunoassay.

Table 1. Patient characteristics

Characteristic	Number of patients
Total patients	43 (80 courses)
Median age 63 (Range (19–75)	
Sex (M/F)	34/9
Prior therapy	43
Radiotherapy alone	8
Chemotherapy alone	12
Chemotherapy and radiotherapy	19
Interferon and radiotherapy	1
None	3
Median performance status (Range)	(0–3)

Table 2. Tumor site (histology)

Lung (non-small cell)	16
Lung (small cell)	1
Renal cell (adenocarcinoma)	6
Colon (adenocarcinoma)	5
Pancreas (adenocarcinoma)	4
Breast (adenocarcinoma)	1
Endometrium (adenocarcinoma)	1
Prostate (adenocarcinoma)	1
Head and neck (squamous)	1
Head and neck (sarcoma)	1
Gastric (adenocarcinoma)	1
Lymphoma (diffuse histiocytic)	1
Femur (osteogenic sarcoma)	1
Thigh (malignant fibrous histiocytoma)	1
Skin (melanoma)	1
Arm (chondrosarcoma)	1

with non-small cell lung cancer was inevaluable for toxicity or tumor response. This patient, treated at 0.63 mg/m^2, died on day 5 of his first course from a massive aspiration related to his tumor-induced bilateral vocal cord paralysis. A second patient treated at 0.63 mg/m^2 died on day 25 of his second course of didemnin B secondary to tumor erosion into his pulmonary artery. Both deaths were felt to be unrelated to treatment. All other patients and courses were fully evaluable for toxicity and response.

Dose-limiting toxicity

Nausea and vomiting were identified as the dose-limiting toxicities in this Phase I study. Table 4 summarizes the incidence of Grade 2 or 3 nausea and vomiting for all patients treated without pretreatment antiemetics. The onset of nausea and vomiting generally occurred during infusion of the drug. Eight patients were treated at the 3.47 mg/m^2 dose level (Table 5). Four patients received four courses with no pretreatment antiemetics and all four experienced Grade 2 or 3 nausea and vomiting.

Four patients received seven courses for which they were pretreated with a vigorous antiemetic regimen (described in Table 5). Only one of these patients experienced nausea and vomiting of similar severity with one course. A single patient treated at 4.51 mg/m^2 was given the same antiemetic regimen but experienced Grade 3 nausea and vomiting which persisted for 2 weeks requiring continued hospitalization with parenteral hydration and antiemetics. Efforts to identify a distinct cause for the nausea and vomiting, including upper gastrointestinal contrast X-rays (UGI) and calcium, creatinine and amylase levels were unrevealing.

Other toxicities

Moderate elevations in hepatic transaminases in the first week following treatment, especially in

Table 3. Procedure for RIA of didemnin B

Reagents	Total counts tube	Volume added (ml) NSB*	Zero tube	Standard or sample tube
Diluent buffer	0.9	0.4	0.3	0.2
Standard or sample	—	—	—	0.1
Antiserum	—	—	0.1	0.1
[^{3}H]Acetyldidemnin B	0.1	0.1	0.1	0.1
Incubate 60 min at 37°C followed by 10 min at 4°C				
Dextran-coated	—	0.5	0.5	0.5
Mix; 10 min at 4°C, mix; 10 min at 4°C, mix; 10 min at 4°C, centrifuge; count 0.8 ml supernatant				

*NSB: non-specific binding.

Table 4. Incidence of moderate to severe nausea and vomiting (without antiemetic pretreatment)

Dose (mg/m^2)	Number of patients at risk	Number of patients with Grade 2 or 3* toxicity (%)
0.14	3	0 (0%)
0.28	3	1 (33%)
0.42	3	1 (33%)
0.63	4	0 (0%)
0.94	4	1 (25%)
1.22	5	1 (20%)
1.58	6	4 (67%)
2.05	3	1 (33%)
2.67	6	2 (33%)
3.47	4	4 (100%)

*WHO criteria.

patients at the higher dose levels, suggested a transient drug-induced hepatocellular injury (Table 6). Elevations of SGOT were from two to four times the baseline value and occurred by day 6 in all patients treated at 1.58 mg/m^2 or higher who developed such elevations. The rise in SGOT in three patients at the first two dose levels was not felt to be treatment related, although in only one patient with progressive hepatic metastases from widely disseminated breast cancer was there an obvious cause for the rise. In all other patients the rise in SGOT was transient, generally returning to baseline or the normal range within 1 week. Elevations of the serum glutamic–pyruvic acid transaminase (SGPT) were similarly two to four times the baseline value with peak levels occurring from 3 to 18 days following treatment. Mild hyperbilirubinemia occurred in one patient at 0.28 mg/m^2, one at 2.67 mg/m^2, and two at 3.47 mg/m^2. The patient at 0.28 mg/m^2 was described above with progressive hepatic metastases from breast cancer. One patient treated at 3.47 mg/m^2 had a baseline bilirubin of 0.6 mg/dl 2 days prior to treatment, 2.1 mg/dl the day following treatment, with a return to normal of 0.6 mg/dl by day 5. In the other two patients there was a gradual rise in serum bilirubin evident by day 7 in both patients with peak levels at days 5 and 15 respectively. In neither patient did the bilirubin return to normal during the 4-week follow-up period. Both of these patients had liver metastases from gastric (2.67 mg/m^2) and colon (3.47 mg/m^2) carcinoma, respectively.

Other abnormalities noted sporadically during the study but not clearly drug related included:

Table 5. Incidence of nausea and vomiting (with and without prophylactic antiemetics)*

Dose (mg/m^2)	No. of patients/no. of courses	Status	Number of courses with Grade 2–3 toxicity (%)
3.47	4/4	Without antiemetic	4 (100%)
3.47	4/7	With antiemetic	1 (14%)
4.51	1/1	With antiemetic	1 (100%)

*Antiemetic regimen = metoclopromide (2 mg/kg IVB), dexamethasone (25 mg IVB) diphenhydramine (50 IVB) pretherapy
= metoclopromide (1 mg/kg IVB), diphenhydramide (25 mg IVB) Q 4 h × 2 post therapy.

†Nausea and vomiting persisted for 2 weeks despite antiemetics.

*Table 6. Incidence of transaminase elevation ≥ Grade 2 toxicity**

Dose	No. at risk	No. of patients with elevations of: SGOT	SGPT
0.14	3	1 (day 13)†	0
0.28	3	2 (days 5, 24)	0
0.42	3	0	0
0.63	4	0	0
0.94	4	0	0
1.22	5	0	0
1.58	6	1 (day 6)	1 (day 13)
2.05	3	0	1 (day 18)
2.67	7	2 (day 3, 4)	2 (days 8, 15)
3.47	8	4 (days 2, 2, 4, 5)	3 (days 3, 3, 8)
4.51	1	1 (day 5)	1 (day 5)

*SGOT >61, SGPT >51.
†Denotes day of peak elevation.

diarrhea in two patients; increased prothrombin time by 3.5 s above control in two patients at 0.42 and 0.63 mg/m^2; and increased fibrin degradation products in one patient. One patient's i.v. infiltrated during the drug infusion without evidence of local tissue reaction.

As didemnin B is administered in a cremophor-containing solution, it was anticipated there might be an allergic reaction during the trial. A single patient treated at 2.05 mg/m^2 experienced chills, diaphoresis, flushing and hypotension during drug infusion of his second course. For a third and fourth course, the patient was pretreated with cimetidine, dexamethasone and diphenhydramine and tolerated therapy with no evidence of a hypersensitivity reaction. No other patient developed anaphylactoid signs or symptoms.

Monitoring for other toxicities demonstrated no evidence of cardiac, renal, bone marrow or ocular effects of the drug. Although most patients received only one dose of didemnin B, eight patients received two courses, five received three courses, one received four courses and one patient received 13 courses of treatment. In none of these patients was there evidence for cumulative or chronic toxicity with repeated dosing. Only one of the patients with apparent liver toxicity was given more than one dose. This patient, treated at 3.47 mg/m^2, had comparable elevations of transaminases with each cycle.

Clinical pharmacokinetics

Information about the pharmacokinetics of didemnin B was derived from study of 16 patients treated from 0.28 to 4.51 mg/m^2. Plasma concentrations relative to the 5-min sampling time point for patients at each dose level are shown in Table 7. Detectable plasma levels were present in only one patient at sufficient time points to generate a plasma decay curve (Fig. 3). This patient's elimination rate constant was estimated by linear regression analysis using the last two data points on the Cp–t plot. The half-life (0.693/k) for this patient was 10.5 min.

Response

One patient with pancreatic cancer maintained stable disease through 12 courses at a dose of 0.28 mg/m^2. After treatment was discontinued at the patient's request, disease stabilization continued for 3 months at which time measurable progression occurred. When re-entered on study at 2.67 mg/m^2 for one course, rapid disease progression continued. There were no complete or partial responses during the trial.

DISCUSSION

While a number of marine natural products with antiviral and cytotoxic activities have been identified [9], didemnin B is the first such agent to enter clinical trials as an antineoplastic agent. It was selected for further development by the NCI based on its novel structure, immunosuppressive activity [10], *in vitro* cytotoxicity and *in vivo* antitumor effect.

The dose-limiting toxicity of didemnin B given as a 30-min infusion at 28 day intervals is nausea and vomiting. The severity of nausea and vomiting appeared to be ameliorated by a combination antiemetic regimen of dexamethasone, metoclopramide and diphenhydramine. This observation as well as the rapid onset of nausea during drug administration suggests that its emetic effect is centrally mediated through the chemoreceptor trigger zone rather than a direct effect on the gastrointestinal tract. Patient tolerance of the drug on this schedule was otherwise quite acceptable with only mild elevations of liver function tests seen in 10 patients. Repeated dosing might allow a higher total dose of the drug to be administered although in a concurrent Phase I trial of didemnin B using a daily

Table 7. Didemnin B: Plasma level at 5 min post infusion

Patient No.	Dose mg/m²	Dose mg	BSA	Infusion duration (min)	Plasma concentration* (Pg/ml)
1	0.28	0.47	1.67	30	—†
2	0.42	0.66	1.56	30	—
3	0.63	1.0	1.65	30	—
4	0.94	1.38	1.47	31	1500
5	1.22	2.27	1.88	30	—
6	1.22	2.56	2.12	35	2000
7	1.58	2.72	1.7	32	—
8	1.58	2.75	1.76	37	—
	1.58	3.16	2.0	30	—
10	2.05	3.98	1.94	30	—
11	2.05	3.83	1.87	51	16,000
12	2.05	4.12	2.01	45	1200
13	2.67	4.43	1.66	31	1100
14	3.47	5.48	1.58	38	3900
	(3.47)	(5.48)	(1.58)	30	2000
15	3.47	6.35	1.80	40	9000
16	4.51	7.94	1.76	35	6600

*All sample times reported were at 5 min post infusion except patient 14 whose sample was drawn immediately at the completion of infusion.
†Non-detectable.
‡Pharmacokinetics obtained on two separate occasions.

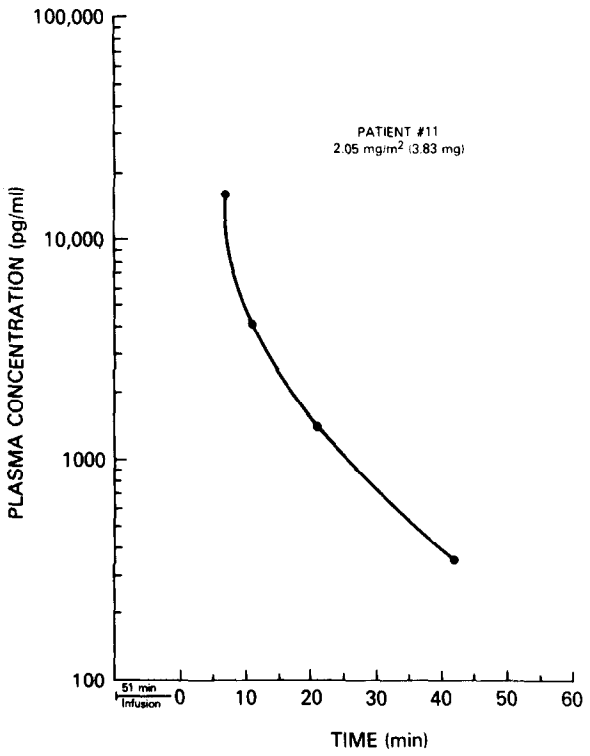

Fig. 3. Plasma decay curve of didemnin B.

times five schedule at the University of Vermont, significant nausea and vomiting have been observed at comparable total dose levels [11].

The non-detectability and rapid disappearance of didemnin B from plasma suggests that the drug is being sequestered or rapidly converted to a metabolite not identified by the radiolabeled antibody. Preliminary evidence in an isolated rat liver perfusion model indicated that didemnin B is extensively metabolized with less than 10% excreted unchanged in the bile [12]. The drug also appears to be partially sequestered with 30% of the drug moving from plasma to the cellular components of blood within 15 min. Nearly 50% of the drug is removed from plasma within 60 min [13]. A combination of both rapid clearance (metabolism/sequestration) and insufficient sampling during and immediately after drug infusion might explain why plasma levels do not clearly increase with dose. On the daily times 5 schedule, peak plasma levels were reportedly very low (100 ng/ml) even after receiving the drug as a 10 min infusion [11].

More data are required to draw any conclusions regarding the pharmacokinetics of didemnin B. Unfortunately the lag time between the completion of the clinical trial and the development of a sensitive assay did not allow for the alteration of our sampling schedule for the optimization of the data. Future considerations include describing the disposition of didemnin B in patients enrolled in phase II clinical trials. The smaller volume requirements with the radioimmunoassay will permit us to increase the number of sampling time points both during and after infusion which should enable the kinetics of didemnin B in both whole blood and plasma to be defined.

The recommended dose for Phase II studies of didemnin B on a single-dose schedule is 2.67 mg/m^2 in 150 cm^3 of normal saline over 30 min without prophylactic antiemetics and 3.47 mg/m^2 if an antiemetic regimen is used. The potential hepatic toxicity of this drug remains to be further defined such that monitoring of liver function tests should be an integral part of Phase II trials.

REFERENCES

1. Rinehart KL Jr, Gloer JB, Wilson GR *et al.* Antiviral and antitumor compounds from tunicates. *Fed Proc* 1983, **42**, 87–90.
2. Rinehart KL Jr, Gloer JB, Hughes RG Jr *et al.* Didemnins: antiviral and antitumor depsipeptides from a Caribbean tunicate. *Science* 1981, **212**, 933–935.
3. Chun HG, Davies B, Hoth D *et al.* Didemnin B: the first marine compound entering clinical trials as an antineoplastic agent. *Invest New Drugs* 1986, **4**, 279–284.
4. Page JG, Hubbard ST, Kastello MD *et al.* Effects of two new antineoplastic agents on blood coagulation (abstract). *Proc Am Assoc Cancer Res* 1985, **26**, 369.
5. Li LH, Timmins LG, Wallace TL *et al.* Mechanism of action of didemnin B, a depsipeptide from the sea. *Cancer Lett* 1984, **23**, 279–288.
6. Crampton SL, Adams EG, Kuentzel SL *et al.* Biochemical and cellular effects of didemnins A and B. *Cancer Res* 1984, **44**, 1796–1801.
7. Jiang TL, Liu RH, Salmon SE. Antitumor activity of didemnin B in the human tumor stem cell assay. *Cancer Chemother Pharmacol* 1983, **11**, 1–4.
8. Rossof AH, Johnson PA, Kimmell BD *et al.* *In vitro* phase II study of didemnin B in human cancer. *Proc Am Assoc Cancer Res* 1983, **24**, 315.
9. Rinehart KL Jr, Shaw PD, Sheild LS *et al.* Marine natural products as sources of antiviral, antimicrobial, and antineoplastic agents. *Pure Appl Chem* 1981, **53**, 795–817.
10. Montgomery DW, Zukoski CF. Didemnin B: a new immunosuppressive cyclic peptide with potent activity *in vitro* and *in vivo*. *Transplantation* 1985, **40**, 49–55.
11. Stewart JA, Tong WP, Hartshorn JN, McCormack JJ. Phase I evaluation of didemnin B (NCS 325319). *Proc Am Soc Clin Oncol* 1986, **5**, 33.
12. Tony WP, Webster LK, Hartshorn JN *et al.* Chromatographic assay for didemnin B and application to pharmacological studies. *Proc Am Assoc Cancer Res* 1986, **27**, 281.
13. Phillips J, Schwartz R, Von Hoff DD. Distribution of didemnin B in blood cells and plasma. Manuscript in preparation.