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Phase I/II clinical trial of didemnin B in non-small-cell lung cancer: neuromuscular toxicity is dose-limiting*

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Summary. Didemnin B (NSC 325319), a cyclic depsipeptide isolated from a Caribbean tunicate, exhibits potent preclinical antitumor activity. In previous phase I studies, 3.47 mg/m^2 was the maximally tolerated dose, with nausea and vomiting being the dose-limiting toxicity. The drug was given in a single bolus infusion over 30 min every 28 days. In the current study, 30 patients presenting with previously treated non-small-cell lung cancer (NSCLC) received 46 courses of the drug at doses ranging from 3.47 to 9.1 mg/m^2. Neuromuscular toxicity was dose-limiting. Nausea and vomiting appeared to be correlated with dose levels and were ameliorated by a combination of antiemetics including dexamethasone. Other side effects included a mild rise in hepatic enzymes and an allergic reaction that was preventable by the addition of corticosteroids to the premedication regimen. In all, 2 minor responses were seen among 24 evaluable patients. Because neuromuscular toxicity is dose-limiting, we recommend that routine measurements of creatine kinase and aldolase, a careful neurologic evaluation, and electromyography and muscle biopsy (if indicated) be incorporated into phase II trials. The recommended dose for phase II studies using a single bolus schedule is 6.3 mg/m^2, following the premedication of patients with antiemetics.

Introduction

The didemnins, a new class of cyclic depsipeptide agents, have been isolated from the Caribbean tunicate (sea squirt) family *Didemnidae* [4, 9, 10]. Three structurally related compounds (didemnins A, B, and C) that differ from each other only in the substituent attached at *N*-methylleucine have been identified [2]. Didemnin B (NSC 325319) is the most potent in terms of inhibition of viral replication, cytotoxicity, and antitumor activity. In vitro, didemnin B is active against L1210 cells, B16 melanoma, P388 leukemia, and KB cells [5]. The drug is a potent inhibitor of protein synthesis and, to a lesser extent, of DNA and RNA synthesis. It also inhibits the progression of B16 melanoma cells from the G_1 to the S phase [2, 6].

Preclinical toxicologic evaluations of didemnin B in CD_2F_1 mice, Fischer 344 rats, and beagles have identified the lymphatics, gastrointestinal tract, liver, and kidneys as the major target organs. In dogs, the inhibition of hepatocyte protein synthesis reduced the production of clotting factors VII, VIII:C, IX, X, and XI [8]; this resulted in dose-related gastrointestinal bleeding due to increased prothrombin time and was the most severe toxicity seen in animal studies.

In a phase I trial, Dorr et al. [3] gave didemnin B as a single intravenous infusion over 30 min every 28 days. The dose-limiting toxicity was nausea and vomiting. Other toxicities were mild, including elevation of transaminases and bilirubin, an increase in prothrombin time in the absence of bleeding, and allergic reaction. Using a dose schedule consisting of five bolus doses given daily, Tong et al. [13] reported that the major toxicities were nausea, vomiting, and hepatotoxicity. Based on these phase I trials, a phase II study has been conducted at The University of Texas M.D. Anderson Cancer Center in untreated colorectal cancer patients at a dose of 3.47 mg/m^2 [1]. This trial produced only nausea and vomiting of grade 2 or less; no hepatotoxicity, coagulopathy, or other dose-limiting toxicity was observed. We therefore designed a repeat phase I study of didemnin B, which was given as an intravenous bolus every 28 days for non-small-cell lung cancer (NSCLC), to redefine the drug's toxicity profile and maximally tolerated dose.

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Table 1. Patients' characteristics

Characteristics		Number of patients (%)
Patients entered		30
Patients evaluable:		
	Toxicity	30 (100)
	Response	24 (80)
Median age (range)	57 (33–73) years	
Sex:		
	M	19 (63)
	F	11 (37)
Performance status:		
	0	3 (10)
	1	19 (63)
	2	8 (27)
Weight loss:		
	<6%	21 (70)
	≥6%	9 (30)
Histology:		
	Adenocarcinoma	20 (67)
	Squamous-cell carcinoma	7 (23)
	Large-cell carcinoma	1 (3)
	Unclassified	2 (7)
Stage:		
	IIIB	5 (17)
	IV	25 (83)
Prior therapy:		
	None	1 (3)
	Chemotherapy	26 (87)
	Radiotherapy only	2 (7)
	Immunotherapy only	1 (3)

Patients and methods

Patients exhibiting histologic proof of NSCLC who were not candidates for treatment with regimens of higher efficacy or priority were eligible for entry into this study. All subjects were required to demonstrate measurable lesions, a Zubrod performance status of ≤2, a life expectancy of ≥12 weeks, adequate bone marrow function (defined as a peripheral absolute granulocyte count of >2,000/mm^3 and a platelet count of >100,000/mm^3), serum creatinine values of <1.5 mg/dl, and bilirubin levels of <1.2 mg/dl. Patients displaying brain metastases were eligible at 3 weeks after the completion of radiotherapy if the brain lesions had become stable or showed regression. Individuals who had received prior chemotherapy or immunotherapy were eligible, provided that they had received no more than one prior regimen, that at least 3 weeks had elapsed since their last treatment, and that they had recovered from all acute toxicities. Written informed consent was obtained from all patients according to institutional guidelines.

The preenrollment evaluation of all patients consisted of a complete medical history and physical examination, tumor measurement, complete blood counts with differential, platelet counts, urinalysis, SMA-12 chemical profile, determination of serum electrolytes, prothrombin and partial thromboplastin times, electrocardiography (EKG), chest roentgenography, computed tomography (CT) of the chest and abdomen, and radioisotope nuclear scanning of bone. CT scanning of the brain and bone marrow aspiration and biopsy were obtained if indicated. As we had observed muscle weakness and myopathy in some patients during the initial part of the study, a full neurologic evaluation, including electromyography (EMG) and measurement of serum creatine kinase (CK) and aldolase levels, was subsequently incorporated into the study.

The formulation of didemnin B used in this study was supplied by the National Cancer Institute's Division of Cancer Treatment (NCI-DCT) as a sterile 1-ml ampule containing 1 mg drug in 5% ethanol, 5% polyoxyethylated castor oil, and 0.9% Sodium Chloride Injection (USP). The calculated dose was diluted in 50 ml normal saline and infused intravenously over 30 min every 28 days. Patients received an antiemetic regimen consisting of 2 mg/kg metoclopramide, 25 mg dexamethasone plus 25 mg diphenhydramine given intravenously at 3, 6, and 9 h following didemnin B administration. In addition, 24 mg dexamethasone was given orally on days 2 and 3, along with supplemental antiemetics, if needed.

The starting dose of didemnin B used in this trial was 3.47 mg/m^2. Three patients were entered at each of the following dose levels: 3.47, 4.2, 4.9, 5.6, 6.3, 7.6, and 9.1 mg/m^2. Additional patients were added at given doses for further definition of toxicity until a side effect of grade 3 or greater was detected in two or more subjects of the six being treated at that dose level. Toxicity was evaluated after each course. Although this was a phase I study, response was also evaluated. Treatment was continued until the occurrence of tumor progression or grade 3 or 4 toxicity. Clinical response and grades of toxicity were defined according to the criteria of the World Health Organization [7].

Results

All 30 patients (Table 1) who were entered in this study were considered to be evaluable for toxicity. The majority were men and exhibited a performance status of 0 or 1. In all, 67% of the subjects had adenocarcinoma; 25 patients displayed stage IV disease. Only 1 subject had not undergone prior treatment; 26 patients had received prior chemotherapy with or without radiotherapy, 2 had undergone radiotherapy alone, and 1 had received immunotherapy.

The hematologic toxicity was mild. A significant case of neutropenia was seen in only one patient treated at a

Table 2. Nonhematologic toxicity over a total of 46 courses

Dose level (mg/m^2)	courses (*n*)	Number of patients according to WHO grade of toxicity										
		Nausea/vomiting			Rise in hepatic transaminase				Neuromuscular toxicity			
		1	2	3	1	2	3	4	1	2	3	4
3.47	3	0	1	0	0	0	0	0	0	0	0	0
4.2	3	2	0	0	0	0	0	0	0	0	0	0
4.9	5	1	1	0	3	0	0	0	0	2	0	0
5.6	6	1	3	0	2	0	0	0	1	2	0	0
6.3	16	2	9	1	7	0	0	0	0	7	3	0
7.6	12	2	6	1	3	1	1	0	1	3	4	1
9.1	1	0	1	0	0	0	0	0	0	0	1	0

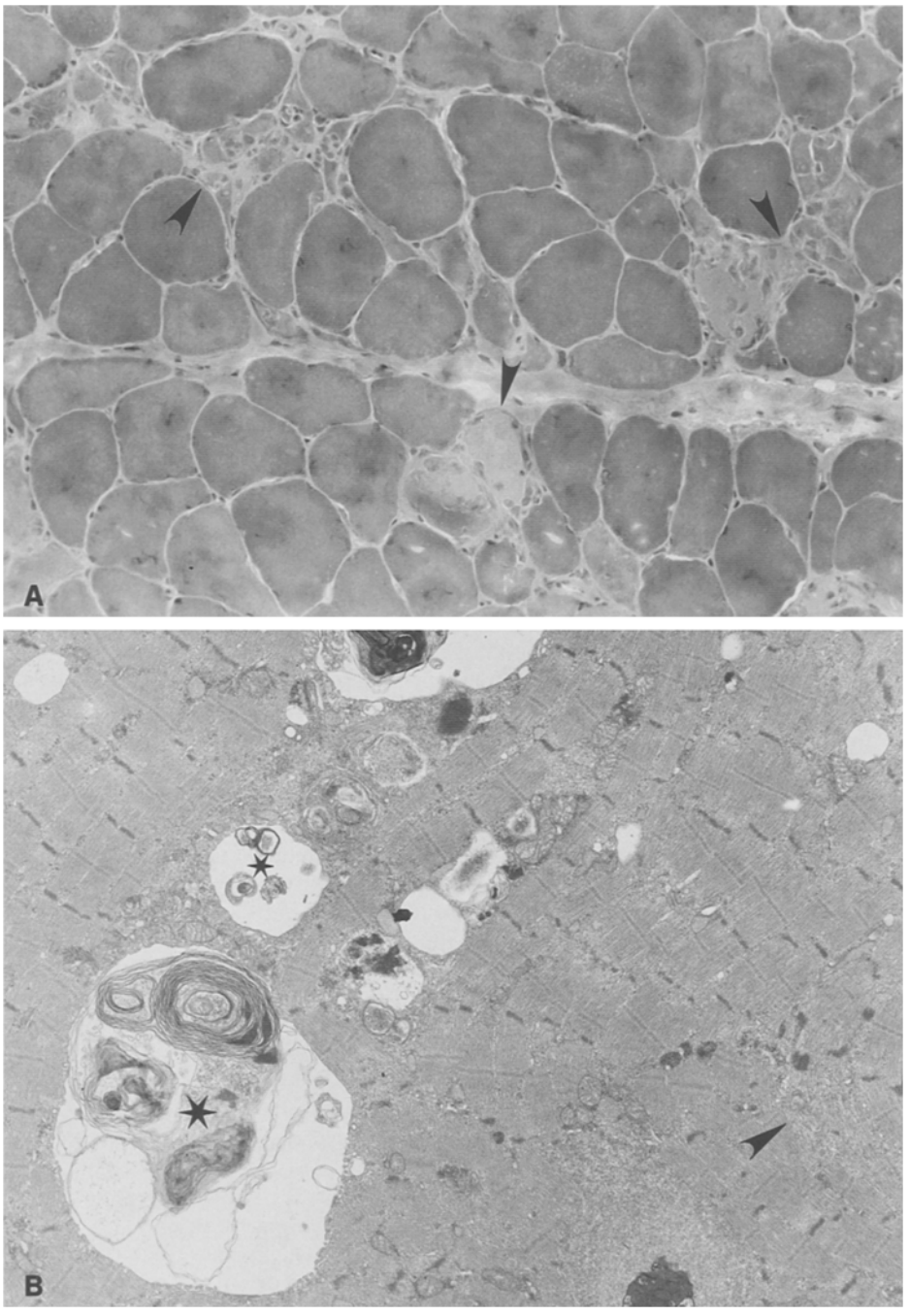
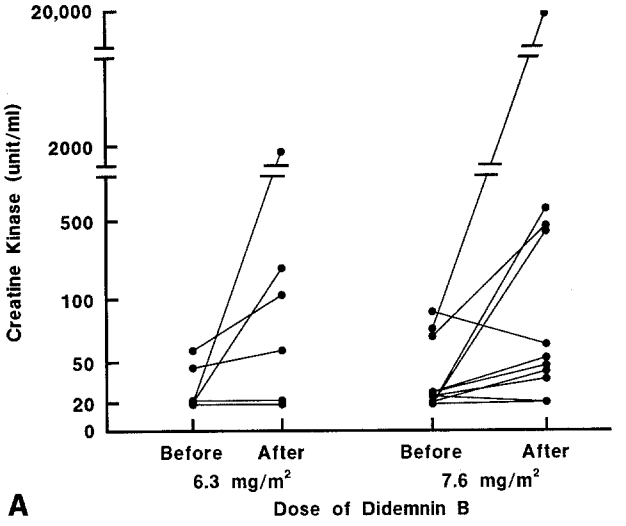


Fig. 1. A Frozen section of gastrocnemius muscle stained with H & E. Note the multifocal groups of necrotic fibers accompanied by regeneration (*arrowheads*). ×220. **B** Electron micrograph of gastrocnemius muscle, showing numerous vacuoles containing degenerate membranous organelles (asterisks), maloriented myofibrils, and focal loss of myofilaments (*arrowhead*). ×2,200

dose level of 6.3 mg/m^2, whose absolute granulocyte count dropped to 800/mm^3 (grade 3) on day 15. Similarly, the only significant case of thrombocytopenia was seen in one patient treated at a dose level of 7.6 mg/m^2, whose platelet count decreased to 42,000/mm^3 (grade 3) on day 12. However, it is noteworthy that this patient's thrombocytopenia occurred in association with hepatorenal syndrome and disseminated intravascular coagulopathy (DIC). We believe that the drop in platelets observed in this subject was more likely due to his DIC rather than to didemnin B toxicity. No significant anemia was seen at any dose level.

The nonhematologic toxicity is outlined in Table 2. At the lower dose levels, nausea and vomiting were mild. At 6.3 mg/m^2, 10 of 16 courses were complicated by grade 2 or 3 nausea/vomiting, and at 7.6 mg/m^2, grade 2 or 3 nausea/vomiting occurred in 7 of 12 courses. Hepatotoxicity was minimal at all dose levels; only one patient treated at 7.6 mg/m^2 exhibited an elevation in transaminase that was 5 times the baseline level. One subject developed an anaphylactic reaction immediately after starting her second infusion of didemnin B at a dose of 3.47 mg/m^2. This patient complained of chest tightness, became cyanotic, and experienced convulsions immediately after the infusion had been started. Fortunately, this anaphylactic reaction reversed soon after the infusion had been stopped. A patient treated at 4.2 mg/m^2 developed a grade 2 skin rash, which also subsided after the completion of the drug infusion. We subsequently added methylprednisone to the premedication regimen and did not observe any further allergic side effects.



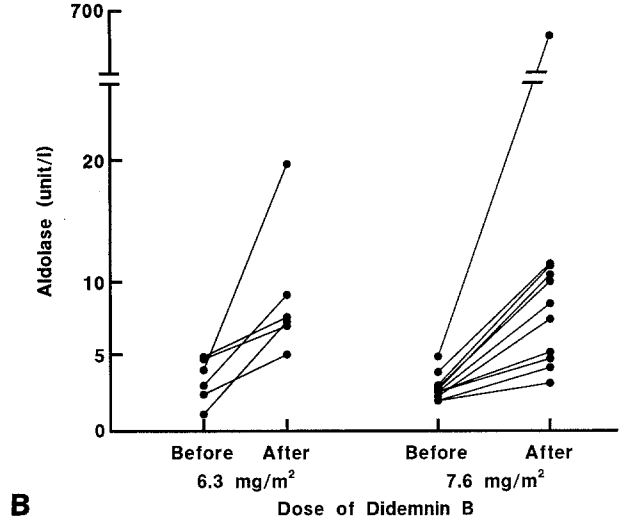


Fig. 2. A Changes in creatine kinase levels before and after treatment with didemnin B. **B** Changes in aldolase levels before and after treatment with didemnin B

Neuromuscular toxicity was dose-limiting (Table 2). At lower dose levels (≤ 4.2 mg/m^2), we did not observe any muscle weakness or myalgia. At 4.9 and 5.6 mg/m^2, grade 2 muscle weakness developed in two of five and two of six evaluable courses, respectively. At 6.3 mg/m^2, grade 2 and 3 weakness occurred in 7 of 16 and 3 of 16 evaluable courses, respectively. Of 12 evaluable courses at 7.6 mg/m^2, didemnin B caused grade 2, 3, and 4 muscle weakness in 3 courses, 4 courses, and 1 course, respectively. One patient treated at this dose developed nearly complete paralysis of all extremities along with severe myalgia. Biopsy of the grastrocnemius muscle of that particular subject revealed a severe degree of polyfocal necrosis and phagocytosis accompanied by regeneration and intense catabolic activity throughout the specimen (Fig. 1A). Fibers exhibiting internal nuclei, cytoarchitectural changes, and increased numbers of fat droplets were also present. However, there was no inflammatory infiltrate. Electron microscopy (Fig. 1B) demonstrated many vacuolated fibers containing granular amorphous material, degenerate membranous organelles, and honeycomb profiles of T-tubules displaying infrequent exocytosis of vacuolar contents. Disorganization of myofibrils, focal loss of myofilaments, and occasional subsarcolemmal accumulation of mitochondria and intranuclear filaments were also seen. Biopsy of the deltoid muscle revealed only severe type II fiber atrophy, which was most probably the result of disuse or a remote effect of the carcinoma. Another patient who developed grade 2 muscular weakness at the 6.3 mg/m^2 dose level also underwent a muscle biopsy, which revealed mild myopathy along with degenerative changes in muscle fibers (data not shown).

Table 3. Neuromuscular toxicity and EMG study in 9 patients

Patient number	Neuromuscular weakness	Didemnin B dose (mg/m^2)	EMG findings (pre- vs Post-treatment)
1	Grade 0	6.3	No follow-up
2	Grade 0	6.3	No follow-up
3	Grade 0	6.3	No follow-up
4	Grade 0	6.3	No follow-up
5	Grade 0	6.3	Myotonia
6	Grade 0	7.6	Myotonia
7	Grade 2	6.3[a]	Myotonia
8	Grade 4	7.6[b]	Myotonia
9	Grade 3	9.1	No change

[a] Muscle biopsy revealed mild myopathy with degenerative changes in single muscle fibers

[b] Muscle biopsy revealed marked multifocal necrosis and atrophic changes with regeneration of muscle fibers

Beginning at the dose of 6.3 mg/m^2, we routinely measured serum CK levels before and after didemnin B treatment (Fig. 2A). At 6.3 mg/m^2, CK values were significantly elevated in three of six patients, all of whom displayed muscle weakness of greater than grade 2; the three subjects whose CK level remained unchanged exhibited no symptoms. Similarly, the majority of patients treated at 7.6 mg/m^2 showed markedly elevated CK levels. For example, one individual who developed grade 4 muscular weakness and severe myopathy as revealed by muscle biopsy exhibited a CK level as high as 19,900 units/ml. As shown in Fig. 2B, serum aldolase levels rose after didemnin B treatment. At 6.3 mg/m^2, four of six patients displayed significantly increased aldolase values (≥ 2 times the baseline level), but this parameter remained unchanged in two subjects after didemnin B treatment. Of 11 patients treated at the 7.6 mg/m^2 dose, 9 also showed a markedly elevated aldolase value (Fig. 2B). It was particularly noteworthy that the same patient who developed grade 4 myopathy at 7.6 mg/m^2 also displayed a serum aldolase level of 615 units/l.

Because of this unanticipated neuromuscular toxicity, EMG was routinely performed in the last nine patients studied. The results are shown in Table 3. Two of six clinically asymptomatic subjects developed mild myotonia at doses of 6.3 or 7.6 mg/m^2; four patients did not undergo follow-up examinations because they either died or were removed from the study before a second EMG was obtained. One individual who developed grade 2 weakness at

6.3 mg/m^2 showed myotonic changes on follow-up EMG. The subject who developed severe myalgia and nearly complete paralysis of all extremities at 7.6 mg/m^2 demonstrated prominent myopathic abnormalities in proximal muscles as determined by EMG examination. The one patient who received 9.1 mg/m^2 didemnin B and developed grade 3 weakness exhibited no neuromyopathic changes on follow-up EMG.

Among 30 patients, 6 were inevaluable for response (1 did not complete the first dose, 3 died prematurely due to rapid progression of disease, and 2 died early of causes unrelated to their disease) and only 2 achieved a minor response at dose levels of 4.9 and 6.3 mg/m^2. In 9 subjects, disease stabilized after 2 courses but then rapidly progressed. The remaining 13 patients exhibited rapid progression of disease after 1 course of treatment. There was no complete or partial response.

Discussion

Didemnin B is the first of a number of natural marine products exhibiting antiviral and cytotoxic activities to enter clinical trials as an antineoplastic agent [4, 9, 10]. In previous phase I trials, the dose-limiting toxicity of didemnin B given as a 30-min infusion at 28-day intervals was severe nausea and vomiting [3, 12]. However, phase II trials [1, 11] failed to show any dose-limiting toxicity. Thus, we repeated a phase I study of didemnin B in NSCLC to redefine its toxicity spectrum and its maximally tolerated dose.

We observed dose-related nausea and vomiting that appeared to be ameliorated by an antiemetic regimen of dexamethasone, metoclopramide, and diphenhydramine. Other toxicities included a mild elevation in hepatic enzymes and minimal myelosuppression, both of which were quite acceptable.

Our most significant finding was the dose-limiting neuromuscular toxicity of didemnin B, a side effect that had not been noted in previous phase I or phase II studies. This neuromuscular toxicity appeared to be dose-related and was characterized by muscle weakness and myalgia in association with elevated serum CK and aldolase levels that correlated well with the degree of symptomatology. We found EMG to be helpful both in determining the nature of the neuropathy or myopathy and in assessing the degree of muscle damage caused by the drug. Muscle biopsies were done in two patients; these demonstrated mild myopathy along with degenerative changes in muscle fibers in a subject who developed grade 2 toxicity and severe multifocal muscle necrosis associated with atrophic changes in a patient who exhibited grade 4 toxicity. There were only 2 minor responses among 24 patients. This is not surprising, since almost all subjects in this trial had previously been treated for NSCLC. It will be important to define the rate of response to didemnin B in patients who have not undergone prior chemotherapy.

We conclude that the dose-related neuromuscular toxicity described herein is the dose-limiting toxicity of didemnin B. The maximally tolerated dose of this drug appeared to be 6.3 mg/m^2, and we recommend that any future phase II trials of didemnin B begin at this dose level. In addition, we suggest that subsequent studies incorporate a careful neurologic examination, the routine measurement of serum CK and aldolase values prior to and after didemnin B treatment, and EMG and muscle biopsies when indicated.

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