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# Phase II clinical and pharmacological study of didemnin B in patients with metastatic breast cancer

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**Summary**

Sixteen evaluable patients with metastatic breast cancer were entered into a phase II trial of didemnin B. They received the drug at an initial dose of 5.6 mg/m$^2$ every 21 to 28 days. Major toxicities noted were myalgia and nausea and vomiting while myelosuppression was mild. There were no complete responses; however, two minor responses were observed. The pharmacokinetics of didemnin B were studied in 10 patients who received the drug as 30 to 60 min i.v. infusions. A sensitive competitive inhibition enzyme immunoassay was used to quantitate didemnin B levels. Drug was observed to be rapidly cleared from plasma in a biphasic manner ($t½\alpha$ = 0.12 hr, $t½\beta$ = 4.8 hr). Although the assay could not identify the presence of specific metabolites, the increase of apparent didemnin B levels in plasma at later time points suggested the formation of unidentified metabolites which cross reacted with the antibody in the analytical procedure. *In vitro* experiments indicated that didemnin B was not bound to bovine serum albumin and only a minor portion (24%) of drug was found associated with red blood cells.

## Introduction

Didemnin B is one of three structurally related cyclic peptides isolated from a Caribbean tunicate [1]. The didemnins, of which didemnin B is the most active, have been shown to possess antiviral [1–3], antiproliferative [4], cytotoxic [1–3] and antitumor activities [2,3,5]. The mechanism of antitumor action of the didemnins is not known, but this family of compounds has been shown to inhibit protein synthesis and to a lesser extent DNA synthesis [6,7]. In addition, low doses of didemnin B can arrest progression of the cell cycle from G1 to S phase, although the drug can lead to death in all phases of the cell cycle.

In previous clinical trials, toxicities of didemnin B included nausea and vomiting [8], elevations of SGOT [8] and neuromuscular toxicity [9] which was considered to be dose limiting. Because of a lack of a sensitive analytical assay, correlations of drug-mediated toxicities with pharmacokinetic parameters were not possible. To determine the clinical pharmacology of didemnin B, we developed a sensitive competitive enzyme immunoassay for this drug. The assay, which was capable of detecting didemnin B in plasma at a lower limit of quantification of 2 ng/ml, was used to investigate the pharmacokinetic parameters of this drug in patients with metastatic breast cancer.

## Materials and methods

Patients considered eligible for this study signed informed consent forms and had failed no more than one regimen for recurrent disease. Patient charac-

*Table 1.* Patient characteristics

| | |
|---|---|
| Number of patients entered | 17 |
| Evaluability status: | |
| Inevaluable | 1 |
| Response & toxicity | 16 |
| Age median (range) | 56 (43–72) |
| Performance status (Zubrod scale): | |
| 0 | 8 |
| 1 | 8 |
| Histology: | |
| Carcinoma with sarcomatoid metaplasia | 1 |
| Duct carcinoma, invasive | 14 |
| Lobular carcinoma, invasive | 1 |
| Prior therapy: | |
| Chemotherapy | 16 |
| Hormone | 8 |
| Radiation | 7 |
| Surgery | 16 |
| Number of prior chemotherapy regimens: | |
| 1 | 14 |
| 2 | 2 |
| Number of prior agents: | |
| 2 | 2 |
| 3 | 11 |
| > 3 | 3 |

teristics are listed in Table 1. Drug was administered in 30 to 60 min infusions and doses were either escalated or reduced based on incidence and/or severity of toxicity. Pretreatment antiemetic therapy consisted of Decadron, Reglan and Benadryl. Reglan and Benadryl were also administered 3, 6, and 9 hr after administration of didemnin B.

Plasma didemnin B pharmacokinetics were determined in 10 patients who received doses of 5.6 mg/m$^2$ (2 courses), 6.3 mg/m$^2$ (9 courses), and 7.5 mg/m$^2$ (2 courses). Blood was collected before infusion, at the end of infusion, and at 0.083, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, and 24.0 hr following infusion. Blood was centrifuged at 1000 × g to obtain plasma which was subsequently packed in dry ice and shipped to Hawaii Biotechnology Group for analysis.

Didemnin B was analyzed by a competitive inhibition enzyme immunoassay; details of the assay have been published recently elsewhere [10]. This assay is capable of detecting didemnin B concentrations in plasma down to 2 ng/ml. BSA-coated microtiter plate wells treated with alkaline phosphatase conjugate and substrate were included on each plate to measure background color development. The mean absorbance at 414 nm of these wells was subtracted from the mean absorbance at 414 of each set of controls and test replicates. Results were recorded as the means of triplicate analyses.

Standard curves were constructed for each analysis using known concentrations of didemnin B. $B/B_0$ values were calculated by dividing the mean of a given set of replicates containing didemnin B inhibitor by the mean of all the wells containing no inhibitor.

*In vitro* experiments were conducted by incubating didemnin B (100 ng/ml) in whole human blood and in 4% BSA in PBS for 4 hr at 37°. The samples in whole blood were centrifuged to obtain plasma. The samples were frozen and shipped to Hawaii Biotechnology Group for analysis as described above.

Plasma didemnin B concentrations were fitted to a two compartment model by nonlinear regression analysis using the RSTRIP Version 4.03 (MicroMath, Inc., Salt Lake City, UT) microcomputer program. Pharmacokinetic parameters were calculated using standard equations [11]; corrections were made for infusion times.

## Results

There were no objective tumor responses observed in this study of the treatment of breast cancer with didemnin B although two minor responses were seen in soft tissue and lymph nodes. Grade 2–3 myalgias or motor deficits lasting from 5 to > 100 days were experienced by 5 of the 16 patients. All patients experienced nausea and vomiting and one had grade 2 diarrhea. Five courses of therapy were associated with grade 1 leukopenia of short duration. Dose limiting toxicity included myalgias of grades 2 (2 patients) and grade 3 (1 patient) and grade 2 motor deficits in 2 patients. Electromyelographic (EMG) studies were, however, normal in all symptomatic patients.

A competitive inhibition enzyme immunoassay was used to quantitate didemnin B in plasma. The assay is based on didemnin A as hapten and is not specific for didemnin B. The plasma clearance of

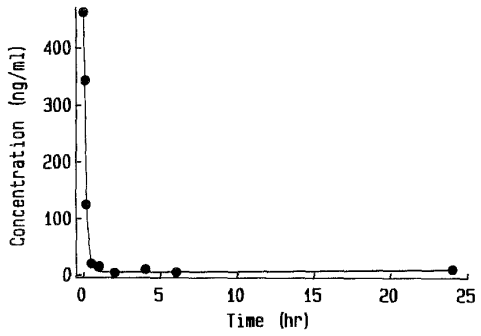


*Fig. 1.* Didemnin B plasma clearance in a patient administered 7.6 mg/m$^2$ of the drug.

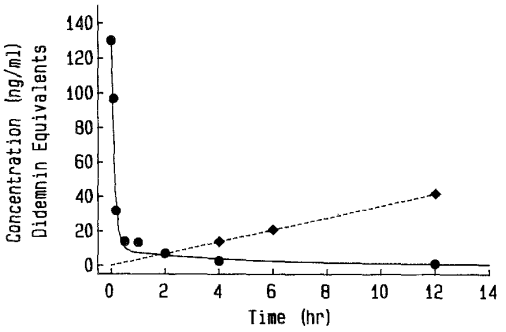


*Fig. 2.* Simulated didemnin B plasma clearance and possible metabolite formation based on feathering technique (see Conclusions). Data are from a patient administered 5.6 mg/m$^2$. Plasma drug levels are indicated by (●) and the formation of possible metabolites are indicated by (◆).

didemnin B was observed to be biphasic (Figs. 1 and 2) with rapid distribution ($t_{1/2}\alpha$ = 0.12 hr) and terminal ($t_{1/2}\beta$ = 4.8 hr) phases. The average pharmacokinetic parameters for the 13 courses were: AUC, 233 ng h/ml; Clt, 29.7 l/h/m$^2$; and Vdss, 57.6 l/m$^2$ (Table 2).

To obtain an estimate of the extent of protein binding and relative distribution in blood cells, drug was incubated for four hr with bovine serum albumin (prepared in phosphate buffered saline) at 37°. Over 95% of the added drug was detectable in the ultrafiltrate suggesting no appreciable protein binding. In addition, the four hr incubation of didemnin B in whole blood resulted in the detection of 76% of the drug, indicating that only 24% of didemnin B was associated with blood cells.

## Conclusions

Figure 1 illustrates the extremely rapid decline of didemnin B during the initial phase of the plasma clearance curve. This precipitous decrease is followed by constant, or even increasing, low levels of drug. The rapid initial clearance may reflect sequestration or metabolism of the drug, either of which may limit clinical effectiveness [5,8]. It has been conjectured, based on *in vitro* stem cell assays, that an AUC of 100–1000 ng h/mL must be achieved for clinical antitumor activity [12]. Our results indicate a mean AUC of 233 ng h/mL (range: 134–462); higher than that expected for activity based on the *in vitro* studies. Since conjugates of didemnin A were used to produce immunogens to elicit antibodies to the ring that is common to the didemnins, it is possible that any didemnin B metabolites possessing the ring structure may also contribute to this value. However, didemnin B is the most active didemnin known and any contribution from a ring containing metabolite would be expected to decrease the observed antitumor effect. Thus, for example, didemnin A, which has 1/10–1/20 [1,6] the cytotoxicity of didemnin B, is also detected by the assay and requires a hundred fold greater dose than didemnin B to achieve comparable antitumor activity against P388 leukemia in mice [1].

The metabolic inactivation of didemnin B is also suggested by animal studies in which didemnin B was only active by ip administration after ip inoculation of tumor [5]. The increase of apparent drug concentrations at later times observed in the majority of courses (9 of 13) we studied suggests possible metabolite formation; in the other instances collection times may not have been long enough to observe increases. Some of these curves could be dissected employing a feathering technique that entailed using the last three concentration-time points to generate a line from which the observed concentrations at earlier times were subtracted (the most outstanding example is shown in Fig. 2). This resulted in two components; the first of which may correspond to the elimination of didemnin B, while the second may indicate the apparent increase of a metabolite.

These simulated drug and metabolite curves indi-

*Table 2.* Pharmacokinetic parameters didemnin B

| Dose ($mg/m^2$) | Ti (hr) | $t½\alpha$ (h) | $t½\beta$ (h) | AUC∞ (ng h/mL) | RT∞ (h) | Clt ($L/h/m^2$) | Vdss ($L/m^2$) |
|---|---|---|---|---|---|---|---|
| 5.6 | 0.73 | 0.08 | 6.2 | 262.0 | 8.3 | 24.1 | 150.0 |
| 5.6 | 0.62 | 0.11 | 3.6 | 193.1 | 3.4 | 29.0 | 52.3 |
| 6.3 | 0.50 | 0.06 | 6.7 | 170.6 | 6.1 | 36.9 | 89.1 |
| 6.3 | 0.50 | 0.08 | 1.3 | 134.0 | 0.5 | 47.0 | 11.0 |
| 6.3 | 0.57 | 0.12 | 3.5 | 195.4 | 3.4 | 32.2 | 66.8 |
| 6.3 | 0.53 | 0.03 | 3.8 | 303.7 | 4.4 | 20.7 | 31.3 |
| 6.3 | 0.50 | 0.51 | 7.7 | 265.7 | 4.3 | 23.7 | 87.7 |
| 6.3 | 0.75 | 0.11 | 9.1 | 221.4 | 8.8 | 28.5 | 109.7 |
| 6.3 | 0.67 | 0.05 | 2.6 | 169.5 | 2.1 | 37.2 | 20.5 |
| 6.3 | 0.40 | 0.09 | 5.3 | 197.6 | 3.6 | 31.9 | 54.6 |
| 6.3 | 0.40 | 0.01 | 5.0 | 216.3 | 2.8 | 29.1 | 37.9 |
| 7.6 | 0.82 | 0.10 | 4.2 | 461.9 | 3.0 | 13.6 | 13.9 |
| 7.6 | 0.92 | 0.06 | 2.9 | 237.9 | 2.8 | 32.0 | 24.5 |
| Median | | 0.12 | 4.8 | 233.0 | 4.1 | 29.7 | 57.6 |
| ± S.D. | | ± 0.12 | ± 2.2 | ± 82.5 | ± 2.4 | ± 8.3 | ± 41.7 |

cate that plasma didemnin B concentrations decrease at a much faster rate than apparent metabolite appears (Fig. 2). This disparity in rates suggests that formation of the putative metabolite is not the major clearance mechanism of didemnin B. The possibility exists that other highly degraded, ring opened metabolites are readily formed. These metabolites would not be detected by the assay and could not be expected to possess cytotoxicity similar to didemnin B. The 'feathering' technique was not applicable to all the data, therefore the pharmacokinetic parameters presented in Table 2 are derived from the actual data points. The incubation of didemnin B with BSA in PBS revealed no appreciable protein binding. This result is in contrast to the report that diacetyldidemnin B is highly bound to human protein [13]. In addition, only 24% of didemnin B was found to be associated with human blood cells versus 45% of diacetyldidemnin B; the greatest uptake of diacetyldidemnin B was by lymphocytes [13]. Significantly, we observed no myelosuppression. These differences between didemnin B and its diacetyl derivative might be attributable to the greater lipophilicity of the diacetyl compound and the resulting increased protein binding and ability to penetrate cell walls.

Thus, the rapid plasma clearance of didemnin B associated with the possible formation of metabolites with less antitumor activity than didemnin B may limit the clinical effectiveness of the drug; there were no objective tumor responses achieved in this phase II clinical study of the treatment of breast cancer with didemnin B.

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