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Inhibition of RNA Viruses In Vitro and in Rift Valley Fever-Infected Mice by Didemnins A and B

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Didemnins, a new class of depsipeptides isolated from a Caribbean tunicate, have been shown to have potent antiviral activity against a broad range of RNA viruses in vitro. Didemnins A and B both protected mice against a lethal challenge of Rift Valley fever virus.

Systematic studies of marine natural products have revealed a number of compounds with antibacterial, antifungal, and antiviral activity (3). Of current interest are the didemnins, a new class of depsipeptides isolated from a Caribbean tunicate (ascidian, sea squirt), which inhibit a number of DNA and RNA viruses (1, 2). Didemnins A and B were shown to inhibit the in vitro growth of herpes simplex virus types 1 and 2 at concentrations of 1.0 and 0.05 μM, respectively. Similar efficacy was demonstrated against three RNA viruses (coxsackievirus A21, equine rhinovirus, and parainfluenza virus 3). We now report results of studies demonstrating the efficacy of the two didemnins against a number of RNA viruses representing families containing highly virulent human pathogens for which no effective therapy or prophylaxis presently exists. We show here the first example of in vivo protection by the didemnins against a lethal challenge with Rift Valley fever virus (RVF).

The antiviral activity of didemnins A and B was studied in vitro with a plaque reduction assay on Vero-76 cells (CRL1587) for RVF (Zagazig 501), Venezuelan equine encephalomyelitis virus (Trinidad donkey), and Pichinde virus AN 3739 and on LLC-MK_2 cells (CCL7) for yellow fever virus (Asibi). Didemnins A and B were dissolved in ethanol, reconstituted with Hanks balanced salt solution, and buffered to pH 7.2 with 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) plus 2% (vol/vol) heat-inactivated fetal bovine serum (4% ethanol, final concentration). Drug solutions (0.3 ml) of appropriate concentration (0 to 2.5 μg/ml for didemnin A and 0 to 0.5 μg/ml for didemnin B) were added to 24-well tissue culture trays (Falcon). Virus (0.1 ml, 50 to 200 PFU) or medium (for tissue culture toxicity controls) was then added. After incubating the trays for 60 min to allow adsorption of the virus, 0.5 ml of overlay medium (basal medium Eagle with Earle salts and 16 mM HEPES, 7.5% [vol/vol] heat-inactivated fetal bovine serum, 0.25% [wt/vol] agarose, 5 μg of gentamicin per ml) was added per well, and the cells were further incubated at 37°C for the time required to yield optimal plaque formation for each virus. Tissue culture plates were then stained, PFU were counted, and tissue culture toxicity was assessed microscopically on the basis of cytopathic effects.

Didemnins A and B were both found to exhibit significant activity against RVF (median inhibition dose [ID_{50}] for didemnins A and B was 1.37 and 0.04 μg/ml, respectively), Venezuelan equine encephalomyelitis virus (ID_{50}, 0.43 and 0.08 μg/ml, respectively), and yellow fever virus (ID_{50}, 0.4 and 0.08 μg/ml, respectively). A concentration of 0.1 μg of didemnin B per ml inhibited plaque formation by these three viruses by more than 80%. Didemnin A, on the other hand, was less efficient, requiring a 25-fold increase in concentration to achieve the same level of virus plaque inhibition. Both didemnins A and B showed mild cytotoxicity in Vero-76 cells as judged by phase-contrast microscopy at concentrations greater than 5 and 0.5 μg/ml (5.3 and 0.45 M), respectively, consisting of increased vacuolation with moderate cell rounding but no detachment. As compared with the other three test viruses, Pichinde virus (ID_{50} for didemnins A and B was 2.9 and 0.22 μg/ml, respectively), a representative arenavirus, was less sensitive to the in vitro antiviral effect of both didemnins.

Studies were also conducted with RVF-infected mice. Female Swiss Webster mice, 3 to 4 weeks of age, were challenged subcutaneously with approximately 250 PFU of RVF per 0.1 ml. Drugs in ethanol (10% final concentration), dissolved in phosphate-buffered sodium chloride (0.15 M, pH 7.2), were administered subcutane-

TABLE 1. Survival of RVF-infected mice treated with didemnin A or B[a]

Mouse group[b]	Treatment regimen		No. of 21-day survivors	Mean no. of days until death (±SD)
	Didemnin	Daily dose (mg/kg)		
Inoculated	None	0	0	3.8 ± 0.8
Inoculated	A	1.25	5	3.8 ± 0.9
		5.0	5	6.0 ± 1.4
		10.0	0	3.7 ± 0.8
Control	A	1.25	10	
		10.0	10	
Inoculated	B	0.1	0	6.2 ± 4.2
		0.15	1	7.0 ± 2.0
		0.2	4	7.0 ± 2.0
		0.25	9	6.0
		1.0	0	2.8 ± 0.6
Control	B	0.2	10	
		0.25	8	10.0 ± 5.7
		1.0	0	2.5 ± 0.6

[a] Didemnins, dissolved in ethanol and reconstituted with Hanks balanced salt solution buffered to pH 7.2 with 10 mM HEPES (4% ethanol, final concentration), were given daily beginning on day −1 for 5 consecutive days.
[b] Ten mice per group, inoculated subcutaneously with 250 to 350 PFU of RVF.

ously for 5 days starting 1 day before virus inoculation. Treatment with didemnin A at a dose of 1.25 to 5 mg/kg per day resulted in 50% survival for mice challenged with RVF (Table 1). Higher dosages given to inoculated but not to control mice appeared to be toxic. Treatment of RVF-infected mice with didemnin B (0.25 mg/kg per day) resulted in a 90% survival rate, although some drug-related deaths were observed. There were no drug-related deaths when the dose was lowered to 0.20 mg/kg, although at this dose the survival rate was decreased to 40%. Didemnin B was toxic and uniformly lethal to mice when administered at 1.0 mg/kg per day for 5 days.

Didemnin B appears to be virostatic or at least to decrease the rate at which viral pathology develops, since all drug-treated RVF-infected mice, including those given the lowest dose, did not die until several days after therapy was discontinued. Didemnin B, therefore, may prove to be more highly efficacious if the treatment period is extended beyond the current 5-day regimen. The activity and effectiveness of the didemnins can be modified, as is clearly evident from the fact that didemnin B is several times more active than the parent compound, didemnin A. The structural complexity of these compounds offers a number of opportunities for chemical modification. If the antiviral action of the didemnins proves to be mediated by a different mechanism(s) than that which causes their toxic effects, then it should be possible to manipulate the chemical structure of the didemnins so as to reduce their toxic properties and yet retain their antiviral activity. Although the therapeutic index of the current derivatives is low, the didemnins may yet prove to be important either alone or in combination with other antiviral agents for the treatment of highly lethal viral infections.

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