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Didemnins A and B. Effectiveness against cutaneous herpes simplex virus in mice

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The antiviral activity of didemnin A and didemnin B against a lethal Semliki Forest virus (SFV) infection of mice and a cutaneous herpes type 1 infection in hairless mice was evaluated. Both compounds significantly decreased the severity of herpesvirus lesions if topical treatment with either didemnin A or didemnin B was started 2 days prior to infection. The survival rate was significantly greater (P=0.03) in the didemnin B treated group than in controls. If initiation of treatment was delayed until 1 h after infection, no activity was obtained. The compounds were not active against cutaneous herpesvirus infection when injected intraperitoneally (i.p.). Didemnin B at concentrations as low as 1.5 μg, administered topically 3 times daily for 5 days, produced skin irritation. Eight times this level of didemnin A could be administered before similar toxicity was observed. The limited activity of didemnins A and B coupled with irritation at the treatment site limits their usefulness in treating cutaneous herpesvirus infection. Neither didemnin A nor B had significant activity in SFV-infected mice.

didemnins A and B; cutaneous herpes simplex virus; Semliki Forest virus; antiviral agents

Introduction

Didemnins are a new class of depsipeptides isolated from a Caribbean tunicate [3]. They inhibit both RNA and DNA viral replication in vitro and are cytotoxic for L1210 murine leukemia cells [4]. Isolation and separation studies have produced compounds, didemnins A, B, and C, that contain both the antiviral and cytotoxic activity [3,4]. In in vitro cytotoxicity and antiviral assays didemnin B was 10–100 times more active than didemnin A [4].

Greater in vivo antitumor activity was seen with didemnin B than A. The dosage level of didemnin B required to increase the lifespan of P388 leukemia-bearing mice by

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40% was 0.06 mg/kg, whereas 8 mg/kg of didemnin A was needed to obtain the same protection [4]. Although both didemnins A and B were ineffective in protecting mice from herpes simplex virus type 1 (HSV-1) encephalitis when both virus and drug were injected intracranially, both compounds protected female mice from genital herpes simplex virus type 2 (HSV-2) infection [2].

In this report, the activity of didemnins A and B in mice infected cutaneously with type 1 herpes simplex virus and lack of activity against a Semliki Forest virus (SMV) infection are described.

Materials and methods

Mice

Upjohn mice Upj:TUC (ICR) spf of random sex and weighing 16 ± 2 g each were used in the SFV studies. They were provided by Upjohn Animal Rearing and Procurement. Female strain HRS/J hairless mice weighing 20 ± 2 g each were used in the cutaneous herpesvirus studies. They were obtained from the Jackson Laboratories, Bar Harbor, ME, U.S.A. Mice were housed under conditions of constant temperature and a 12-h light cycle with food and water available ad libitum.

Virus and method of infection

SFV was originally obtained from S. Baron (National Institutes of Health) and was propagated in weanling ICR mice. The pool used in these studies titered 8×10^8 plaque forming units (PFU)/ml on primary chicken embryo cells. Mice were injected intraperitoneally (i.p.) with approximately 150 PFU. HSV-1/(HWC strain) was originally isolated from a herpetic lip lesion [6]. The virus was propagated in primary rabbit kidney cells and had a titer of 5×10^7 PFU/ml. Mice were infected with 1×10^6 PFU applied with a cotton swab to the lumbar region of hairless mice that had been lightly scratched with a hypodermic needle.

Drug preparation

Didemnins A and B used for antiviral testing against SFV were prepared in 2% dimethylsulfoxide (DMSO). Drug samples applied in the cutaneous herpesvirus studies were prepared using a formulation which consisted of 5% DMSO. 10% Emulphor EL-620, and 85% water.

Treatment

Didemnins A and B were administered i.p. in studies involving SFV. When tested against cutaneous herpesvirus infection, the didemnins were administered either topically at the infection site or i.p.

Evaluation

Progress of infection in hairless mice infected cutaneously with HSV-1 was scored by estimating severity and extent of the lesion using a scale from 0 to 4 [6]. Lesions and deaths in treated and control groups were scored daily. The lesion scores were analyzed at each day post-infection for each group using a Ruskal–Wallis statistic corrected for ties [1]. Survival curves were compared using the computer program developed by Thomas et al [5].

Results

Toxicity studies

Varying concentrations of compound were administered to the skin of normal hairless mice in the lumbar region three times daily for a total of 5 days. Mice were observed daily for toxicity. Mice receiving 13 μg of didemnin A three times daily for 5 days developed local inflammation. Cutaneous toxicity was not observed when a concentration of 6 μg was administered three times daily for 5 days. Didemnin B was more toxic. Levels as low as 1.5 μg administered three times daily caused local skin irritation. At the 13- and 6-μg levels, edema was evident and ended in skin sloughing.

Cutaneous herpes simplex virus infection

Experiments were initiated to evaluate the antiviral activity of didemnins A and B against cutaneous herpes simplex infection. Groups of 10 mice were infected with HSV-1 cutaneously at the lumbar region. Lesions were scored daily using a scale from 0 to 4. Three treatment regimens were evaluated. Results included average daily lesion score, total number of mice that died in each group by the termination of the experiment (19 days), and the mean standard time (MST) of death. Statistical analysis of this data indicated a significant decrease in average lesion score on days 5, 6, 7, and 8 post-infection if treatment was started 2 days pre-infection (Fig. 1). No significant difference was observed between the treated and control groups by 9 days post-infection. No delay in lesion development was observed with didemnin A or B if topical treatment was started at 1 h post-infection.

Neither treatment schedule was effective when didemnin A was administered i.p.

Figure 2 presents standard survival curves for mice treated with didemnin B when treatment was started 2 days pre-infection. Statistically the survival rate was better in the didemnin B-treated animals than in controls ($P=0.03$) if treatment was begun prior to infection, but not if delayed until after infection.

No difference was observed in the survival rate of mice treated i.p.

Semliki Forest Virus infection

To determine if either didemnin A or B had antiviral activity against a lethal SFV

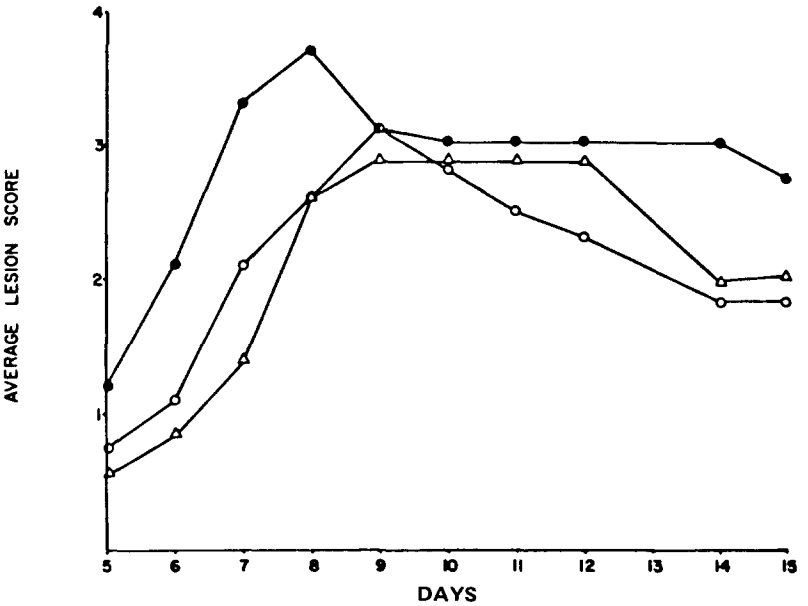

Fig. 1. Effect of didemnins A and B on cutaneous herpes simplex infection in hairless mice. Mice were treated topically (three times daily for 5 days starting 2 days pre-infection) with either didemnin A (○) at a concentration of 6 μg/treatment or didemnin B (△) at a concentration of 0.75 μg/treatment. Control = ●.

infection, groups of mice were treated with either compound at 44, 26, 19, and 3 h before and 3 h after i.p. infection with approximately 15 PFU of SFV. Didemnin A was inactive against SFV when administered at a concentration of 2 mg/kg per day. Didemnin B, when administered at a concentration of 1 mg/kg per day, led to death of about 50% of the mice. At a tolerated dose of 0.5 mg/kg per day no activity was observed with didemnin B.

Conclusion

Studies described in this report were initiated to determine if didemnins A and B would protect mice against either a lethal SFV infection or a cutaneous herpesvirus infection in hairless mice. Although no activity was obtained with either of these compounds in SFV-infected mice, a significant delay in lesion severity was observed in mice infected cutaneously with HSV and treated topically with either didemnin A or B. Treatment had to be initiated 2 days pre-infection and continued three times daily for a total of 5 days for efficacy. The difference in lesion score between treated and control animals was observed only for the first 8 days of infection. Thereafter, no significant difference in lesion score between treated and control animals was observed. An increase in the survival rate of didemnin B-treated mice was obtained only if treatment was started 2 days pre-infection. No activity was observed if treatment was

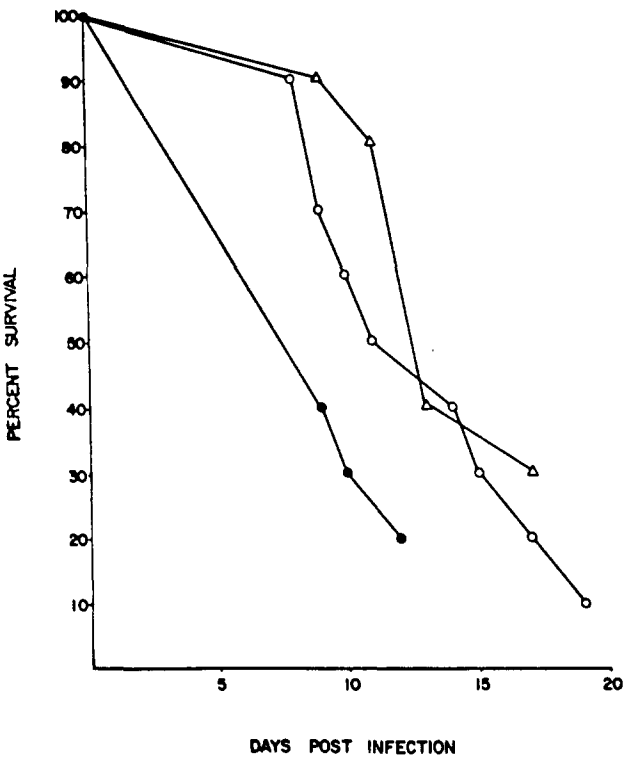

Fig. 2. Survival of hairless mice infected cutaneously with herpes simplex virus and treated (three times daily for 5 days starting 2 days pre-infection) topically with either didemnin A (○) at a concentration of 6 μg/treatment or didemnin B (△) at a concentration of 0.75 μg/treatment. Control = ●.

delayed until 1 h after infection. When didemnin A was administered i.p., no activity was obtained regardless of the treatment schedule.

Relatively low levels of didemnin A or B caused local skin irritation following topical administration. Didemnin A was toxic at levels as low as 13 μg given three times daily for 5 days, and didemnin B was toxic at 1.5 μg using the same treatment regimen.

Although continuing treatment for a longer period of time after infection may increase the activity of didemnin A or B, data presented in this report indicate that, compared to other antiherpes agents, activity was limited. This limited activity along with the degree of skin irritation limit the usefulness of the didemnins in the treatment of cutaneous HSV infections. The antiviral activity of these compounds does not extend to SFV.

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