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Treatment of acyclovir-unresponsive cutaneous Herpes simplex virus infection with topically applied SP-303

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Abstract

The naturally occurring polyphenolic biopolymer SP-303 has in vitro activity against both HSV-1 and HSV-2, including strains that are resistant to acyclovir. Nine AIDS patients with acyclovir-unresponsive mucocutaneous herpes simplex virus infection were treated with thrice daily topical SP-303T ointment in an open-label pilot study. Although a transient decrease in lesion size was observed in 4 patients during study drug therapy, and 3 patients sustained a quantitative decrease in virus burden, neither complete healing nor cessation of virus shedding occurred in any patient. Seven patients complained of pain or burning upon application of the study ointment, causing 1 patient to terminate the study. In summary, application of SP-303T ointment effected no significant improvement in the clinical course of 9 AIDS patients with acyclovir-unresponsive HSV infection.

Keywords: Herpes simplex; Acyclovir-resistance; SP-303; Topical; AIDS

1. Introduction

Therapies for immunocompromised patients with acyclovir-resistant herpes simplex virus (HSV) infection have been under investigation during the past decade. The first

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report of successful treatment of such patients with intravenous foscarnet appeared in 1987 (Vinckier et al., 1987). However, the potential toxicities of foscarnet, its' expense, and the occasional occurrence of foscarnet resistance in patients exposed to the drug repeatedly or chronically (Safrin et al., 1994) suggest that continued investigation of other alternatives is warranted.

SP-303 is an investigational antiviral agent isolated from the latex of the plant *Croton lechleri* (Barnard et al., 1993a). The drug substance is a phenolic polymer of molecular weight 2100 daltons, the monomers of which are the 4 related catechin and gallocatechin isomers. SP-303T is an ointment formulation intended for topical administration which contains 15% of the active ingredient SP-303.

SP-303 has been shown to have in vitro activity against a broad range of viruses, including respiratory syncytial virus, influenza A and B, parainfluenza types 1 and 3, and hepatitis A (Barnard et al., 1993b, Gilbert et al., 1993, Wyde et al., 1993). Additionally, SP-303 possesses in vitro activity against herpes simplex virus (HSV) types 1 and 2, retaining activity against thymidine kinase-negative mutants, and appears to act through inhibition of virus penetration into cells (Barnard et al., 1993a, Safrin et al., 1993). In a recent study, the concentration of SP-303 required to inhibit HSV growth in vitro by 50% or greater (ID_{50}) ranged from 0.9 to 2.1 μg/ml for acyclovir-susceptible strains and from 0.8 to 3.2 μg/ml for acyclovir-resistant, foscarnet-susceptible clinical HSV isolates (Safrin et al., 1993).

In vivo activity against HSV was demonstrated following topical, oral and intraperitoneal administration in murine and guinea pig vaginal models (unpublished data; Shaman Pharmaceuticals Inc.). In one study, topical therapy of mice with 10% SP-303 resulted in a significant reduction in mean lesion score without detectable toxicity (Barnard et al., 1993a). Toxicity studies using oral SP-303 in rats, mice, dogs, and Rhesus monkeys for 5 to 30 days demonstrated variable decreases in body weight gain, pigmentation in intestinal histiocytes and hepatic sinusoidal cells, and/or loose stools (unpublished data; Shaman Pharmaceuticals Inc.). Administration of oral SP-303 to healthy adult male volunteers was associated with asymptomatic elevations in hepatic transaminase levels in one of six studies (unpublished data; Shaman Pharmaceuticals Inc.). Acute dermal irritation tests on intact and abraded skin in rabbits with either SP-303 or SP-303T did not cause irritation.

We evaluated the efficacy and safety of topically applied SP-303T for the treatment of acyclovir-unresponsive cutaneous HSV infection in patients with AIDS.

2. Materials and methods

HIV-infected men or nonpregnant women with a diagnosis of AIDS (Centers for Disease Control, 1987) who had culture-proven cutaneous HSV lesions persisting for ≥ 30 days despite treatment with either oral (≥ 1000 mg/d) or intravenous (≥ 15 mg/kg/d) acyclovir for ≥ 10 days were eligible. Patients with allergies to citrus fruits, black currants, or rosehips were excluded, as were those using concurrent agents with anti-HSV activity or unapproved investigational drugs. Two patients were permitted to remain on the investigational agent d4T (Stavudine®: Bristol Meyers Squibb) while

receiving SP-303T. Other exclusion criteria included an absolute neutrophil count $< 750/\mu l$, platelet count $< 50\,000/\mu l$, hematocrit $< 25\%$, serum transaminase elevations > 3 times the upper limit of normal, total bilirubin ≥ 2.0 mg/dl, or serum creatinine ≥ 2.0 mg/dl. Informed consent from each patient was obtained prior to initiation of study drug therapy.

The SP-303T study ointment was formulated in U.S.P. Hydrophilic Ointment under aseptic conditions at each of the 3 study sites, according to the manufacturer's directions. Investigators and patients were instructed to thinly cover the entire area of all HSV lesions with the ointment thrice daily for a minimum of 14 days. The use of wound dressings was left to the discretion of the investigator and this information not recorded. The first dose of SP-303T was applied by the investigator, followed by a mandatory 1 h period of observation for erythema or pain. Patients self-applied the ointment thereafter, and recorded the times of applications daily in a diary. The minimum duration of therapy was 14 days; patients who demonstrated partial rather than complete healing at that time were permitted to receive extended treatment for a maximum of 42 days. Clinical evaluations were performed on days 1, 4, 7, 10 and 14 of therapy, as well as weekly during extended therapy and at 1 and 2 weeks following cessation of therapy. Systemic toxicities were monitored by serum hematologic and chemistry evaluation and urine analysis on days 1, 4, 7, once-weekly during further treatment, and at each follow-up visit.

Clinical efficacy parameters included serial measurements of lesion size (length $\times$ width), the presence of re-epithelialization, degree of erythema, degree of pain, and the occurrence of new lesions. Treatment with SP-303T of satellite lesions that arose during therapy was permitted. The severity of pain caused by HSV lesions was rated serially,

Table 1
Results of therapy with SP-303T

Pt #	Days of treatment	Total lesion area (mm^2)			Occurrence of new lesions (day)	Reason for discontinuation of therapy
		At study entry	On day 14 (% change)	At end of therapy (% change) *		
1	41	945	945 (0)	330 (−65)	yes (21)	maximum allowable days of therapy
2	14	40	100 (+150)	same	no	enlargement of lesion
3	14	126	101 (−20)	same	no	minimal decrease in lesion size
4	14	323	374 (+16)	same	yes (7,30)	enlargement of lesion
5	22	2100	1800 (−14)	4550 (+117)	no	enlargement of lesion, noncompliance
6	29	561	538 (−4)	2192 (+291) **	yes (4)	enlargement of lesions
7	25	5200	5200 (0)	5200 (0)	yes (21)	daily fevers
8	10	1060	1060 (0) ***	same	yes (7,10)	pain upon application of study drug
9	14	250	900 (+260+)	same	yes (7)	enlargement of lesions

* Percent change is calculated in reference to lesion size at study entry.
** 11 of 12 baseline lesions had coalesced into 4 lesions, leaving a total of 6 discrete lesions.
*** Represents the lesion area on day 10, the last day of therapy for this patient.
+3 of 3 baseline lesions had coalesced into a single lesion.

using a self-administered 10 cm visual analogue scale in a daily diary. Serial photographs of the lesion(s) were obtained for documentation.

Virologic cure was defined as cessation of virus shedding on two or more consecutive cultures. Specimens for herpes culture were obtained at each clinic visit using a Viral Culturette® (Becton Dickinson Microbiological Systems, Cockeysville, MD). A second swab from the lesion was frozen at −70°C for quantitative virus titering: thawed frozen samples were placed in minimum essential media (MEM) with 2% heat-inactivated fetal bovine serum (HIFBS), penicillin, streptomycin, and amphotericin B. The vortexed specimens were sterilized using 0.45 μc syringe filters, diluted serially by 1:10, and added to confluent Vero cells in 6-well plates. Following incubation at 37°C for 60 min, the wells were overlaid with MEM, 5% HIFBS, penicillin, streptomycin, and 0.5% agarose. Plaques were stained with neutral red dye after 5–6 days of incubation and counted manually (Spruance et al., 1984).

The first and last recovered HSV isolate from each patient were tested for susceptibility to acyclovir and SP-303 using the plaque reduction assay in Vero cells (Safrin et al., 1994). However, SP-303 was added simultaneously with virus inoculation, rather than 1 hour later as with acyclovir, in order to allow for the presence of the drug during virus attachment.

3. Results

3.1. Study population

Ten HIV-infected men completed screening evaluations and received treatment with SP-303T; a single patient who was ineligible due to failure to isolate HSV in culture is excluded from this analysis. The median age of the remaining 9 patients was 36 years (range, 25–63), and the median CD4 cell count was 7/mm^3 (range, 2–77).

The median number of lesions at study entry was 1 (range, 1–12). Lesions were perirectal in 6 of the 9 patients; the remaining 3 patients had lesions on the finger, ear and nose, respectively. HSV lesions had been unresponsive to therapy with oral acyclovir for a median of 1.5 months (range, 0.75–6.25). None of the patients had received intravenous acyclovir for the current episode of HSV, although 8 days of intravenous foscarnet had been administered in patient #8 and 21 days of topical trifluridine in patient #4, without healing. Four patients (#2,3,5,6) had a history of prior episodes of acyclovir-unresponsive mucocutaneous HSV, which had healed upon administration of intravenous foscarnet.

3.2. Clinical response

Nine patients received therapy with SP-303T for a median of 18 days (range, 10–41). Study drug therapy was discontinued due to enlargement of HSV lesions in 5 patients, lack of continued healing in 1 patient, adverse experiences in 2 patients, and receipt of the maximum allowable duration of therapy in 1 patient (see Table 1). No patient had complete healing of HSV lesions while on study drug therapy.

The total area of HSV lesions at baseline varied from 40 to 5200 mm^2 (median, 561). On day 14, the median change in lesion size at day 14 was 0, and ranged from a 20% decrease to a 160% increase (see Table 1). Total lesion area on day 14 ranged from 100–5200 m^2 (median, 719). During the initial 14 days of therapy, 3 patients (#3,5,6) had a decrease in total lesion area (by 20, 14, and 4%, respectively), 3 patients (#2,4,9) had an increase in lesion area (by 150, 16, and 260%), and 3 patients had no change in lesion size (see Fig. 1 and Table 1).

Of the 4 patients who continued study drug therapy beyond 14 days, one (patient #1) sustained a decrease in total lesion area, by 65%. Of interest is that the lesion in this patient decreased by 97% on day 28 of therapy (i.e., from 945 to 25 mm^2), but enlarged to 375 mm^2 by day 42, in concert with noncompliance with study drug application. In patient #7, lesion size remained unchanged, and in patients #5 and #6 lesions had enlarged by 117% and 291%, respectively (see Table 1). Patient #5 admitted to noncompliance, missing a total of 10 applications of the drug during the final 6 days of therapy.

Six patients (#1,4,6,7,8,9) developed new HSV lesions during study drug therapy. Lesions were in contiguous sites in 5 patients and in two non-contiguous sites in one.

The median on-study pain score was lower than the baseline score for 1 patient, higher for 2 patients, and unchanged in 4. In no patient did pain resolve completely. Two patients failed to complete a diary, rendering impossible a serial assessment of lesion pain.

3.3. Virologic response

All patients had positive cultures for HSV-2 at baseline. No patient demonstrated sustained cessation of virus shedding during therapy; however, 3 patients became

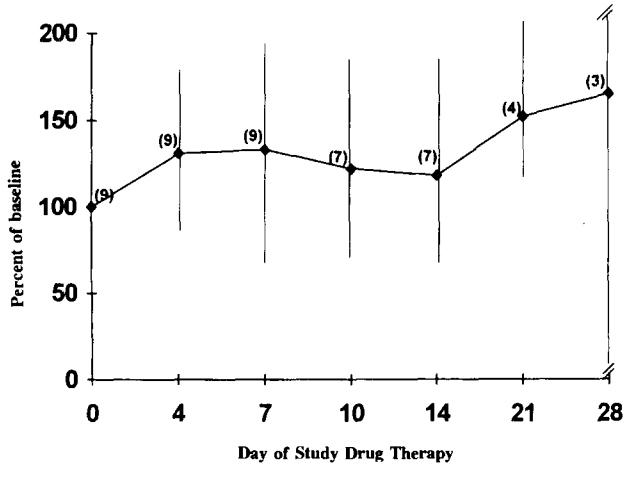

Fig. 1. Mean lesion area during study drug therapy. Lesion area was measured in units of mm^2. Numbers in parentheses represent the number of patients evaluated at each time point. Vertical bars represent one standard error of the mean. Double slashed lines signify that the value is off the scale of the graph.

culture-negative at some point during the study. Virus cultures were transiently negative in patient #1 on day 28 and 35 of therapy (corresponding to near healing of the perirectal lesion) but became positive again on day 41, coincident with lesion enlargement and noncompliance (see above). Patient #2 was culture-negative on day 7, but culture-positive on days 10 and 15 despite continued use of the drug, concurrent with lesion enlargement. Patient #9 was culture-negative on day 14 but discontinued the study on that day due to progressive enlargement of the lesion.

Viral isolates from the first and last days of therapy were analyzed for in vitro susceptibility to acyclovir and SP-303. Baseline ID_{50} values for SP-303 ranged from 6.7 to 14 μg/ml; final ID_{50} values ranged from 3.7 to 12.5 μg/ml. In no patient did SP-303 ID_{50} values rise substantially to indicate the emergence of resistance. Eight of 9 HSV isolates were resistant to acyclovir in vitro at the time of study entry (ID_{50} range, 11.5 to 92 μg/ml) and at the completion of therapy (ID_{50} range, 5.2 to 300 μg/ml). Patient #8 had acyclovir-susceptible isolates recovered both at study entry and at the completion of therapy (ID_{50}, 1.2 and 0.9 μg/ml, respectively), despite demonstrated lack of response to oral acyclovir in doses of 4000 mg daily for 3 weeks.

The median virus titer at the time of study entry was 725 pfu/ml (range, 130 to 84 000) in the 8 patients for which this data was available. Comparison of virus titers at the completion of therapy with that at entry showed a decrease in 3 patients (by 86, 92 and 96%, respectively) and an increase in 4 patients (by 45, 154, 183, and 614%, respectively). The median on-therapy virus titer averaged 175% that at study entry (range, 14–714%; see Fig. 2). Change in virus titer did not significantly correlate with change in lesion area (Spearman correlation coefficient, 0.09; $P = 0.8$).

3.4. Safety

Two patients (22%) withdrew from the study due to an adverse experience: patient #8 complained of severe burning upon application of SP-303T ointment, and patient #7

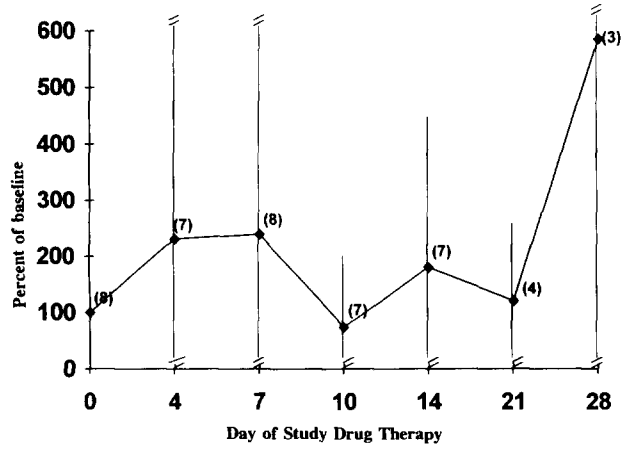

Fig. 2. Mean virus titer during study drug therapy. Virus titers are in units of PFU (plaque-forming units)/ml. Numbers in parentheses represent the number of patients evaluated at each time point. Vertical bars represent one standard error of the mean. Double slashed lines signify that the value is off the scale of the graph.

had self-reported fevers to 103°F which reportedly receded following discontinuation of the study drug. An additional 6 patients (#1,3,4,5,6,9) described a burning or dull aching sensation associated with application of the study medication; in many patients this was occasional rather than constant. A single patient (#4) had mild erythema possibly associated with study drug application, which was not dose-limiting.

Two patients (#1,2) complained of nausea throughout the study, in both instances felt by the investigator to be unrelated to the study medication. No patient exhibited diarrhea, elevation in serum liver function tests, or evidence of substantial laboratory abnormalities on serial hematologic and chemistry serum determinations.

Two patients developed concurrent illnesses during the study. Patient #1 began therapy with ganciclovir on day 30 due to a relapse of cytomegalovirus retinitis, and patient #5 began therapy for *M. avium* complex bacteremia on day 4.

4. Discussion

We evaluated 9 AIDS patients in a pilot investigation of the efficacy and safety of topically applied SP-303T for the treatment of cutaneous acyclovir-unresponsive HSV infection. There was a varied response to study drug therapy, with 3 of 9 patients manifesting a decrease in lesion size during the initial 14-day study period but in 3 others an increase in size during that interval noted. One patient achieved near-complete healing on day 28 of therapy, with regrowth of the lesion associated with noncompliance during the remaining 13 days of therapy. Although 3 of 8 patients sustained a decrease in virus titer during therapy, the remaining 5 had increases in virus burden as measured by this technique. In no patient was either complete healing or virologic cure achieved.

The worsening of several lesions despite lack of evidence for the emergence of in vitro resistance to SP-303, as well as the varied responses of our patients to application of the study drug, suggest that a multitude of factors may interact to determine the success of topical treatment of acyclovir-unresponsive HSV infection. Possible explanations for the observed lack of clinical or virologic effect of SP-303T in this study include: inadequate release of the compound from the formulation, inadequate penetration of this large molecule into infected tissues, and/or inability of SP-303 to incur healing of already existing herpetic lesions, due to its mode of action of inhibition of virus penetration into cells. As in previous studies (Safrin et al., 1991, Safrin et al., 1994), it should be noted that the advanced degree of immunosuppression, as well as the presence of concomitant, debilitating AIDS-related conditions in many patients with acyclovir-unresponsive HSV infection renders the treatment of this condition difficult. Nevertheless, the reported success of certain topical therapies in sporadic patients, such as trifluridine (Murphy et al., 1992), combined trifluridine with interferon-α (Birch et al., 1992), and HPMPC (Snoeck et al., 1993), indicates that continued investigation of new therapies with topical formulations may be a worthwhile pursuit.

The bioflavonoid SP-303 is the first antiviral agent acting by inhibition of virus penetration to be studied as monotherapy for acyclovir-unresponsive HSV infection. The small number of patients studied makes definitive assessment of the utility of this formulation difficult. Although the drug appeared to be devoid of systemic toxicity in

this study, application of SP-303T was associated with a burning or painful sensation in 7 of the 9 patients, resulting in discontinuation of therapy in one. It is unclear whether this reflects irritation of pain receptors by the drug, or by a component of the ointment. Additional studies using a different formulation may be warranted.

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