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Topical Treatment of Infection with Acyclovir-Resistant Mucocutaneous Herpes Simplex Virus with the Ribonucleotide Reductase Inhibitor 348U87 in Combination with Acyclovir

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The thiocarbonohydrazone 348U87 inactivates herpes simplex virus ribonucleotide reductase and potentiates the activity of acyclovir against wild-type and acyclovir-resistant strains of herpes simplex virus. We treated 10 human immunodeficiency virus-infected patients with acyclovir-resistant anogenital herpes simplex virus infection with a topical preparation of 348U87 (3%) in combination with acyclovir (5%) in an open-labelled study. Transient improvement with combination therapy occurred frequently; however, target lesions reepithelialized completely in only 1 of 10 patients. Termination of study drug therapy was most often due to cessation of therapeutic effect before complete resolution of lesions. As currently formulated, topical 348U87 offers little therapeutic benefit for this indication.

Ribonucleotide reductase is a key enzyme in the synthesis of viral DNA, catalyzing the conversion of ribonucleotides to deoxyribonucleotides (1). The herpesvirus-encoded ribonucleotide reductase is not essential for growth in tissue culture or establishment of latency (16); however, the enzyme appears to be essential for pathogenicity in mice (1, 6). Therefore, the enzyme has been pursued as a possible target for antiviral chemotherapy.

Herpes simplex virus (HSV) encodes a ribonucleotide reductase that is biochemically and immunologically distinct from the cellular enzyme (16). The thiocarbonohydrazone class of irreversible ribonucleotide reductase inhibitors has a substantially higher affinity and inhibitory effect against the viral enzyme than against the mammalian counterpart (13). In cells treated with the thiocarbonohydrazone A1110U (2-acetylpyridine-5-[(dimethylamino)thiocarbonyl]thiocarbonohydrazone) and acyclovir, intracellular pools of acyclovir triphosphate are increased as dGTP levels are reduced, thus potentiating the effects of acyclovir (13).

The addition of A1110U to acyclovir results in a decrease in the 50% inhibitory concentration of HSV types 1 and 2 (HSV-1 and HSV-2, respectively) and varicella-zoster virus to acyclovir without significant toxicity to the host cells (13). In athymic and hairless mice infected intracutaneously with wild-type or thymidine kinase (TK)-deficient HSV-1, the combination of topical or oral acyclovir with topical A1110U was significantly more effective in the reduction of cutaneous lesions than treatment with either drug alone (3). In dorsally infected athymic mice, topical treatment of zosteriform rash with combined A1110U and acyclovir was significantly more effective in reducing the scores of lesions caused by wild-type HSV-1, wild-type HSV-2, TK-deficient HSV-1, and TK-altered HSV-1 mutants than use of either drug alone (7). The results of combination therapy against lesions caused by a DNA polymerase HSV-1 mutant also were additive but did not result in statistically significant synergy (7).

The compound 348U87 (2-acetylpyridine-5-[2-chloroanilino-thiocarbonyl]thiocarbonohydrazone) potentiates the antiviral activity of acyclovir as effectively as A1110U but is devoid of hematological toxicity (14, 15). The addition of 0.8 μM 348U87 to acyclovir in tissue culture resulted in a decrease of the 90% inhibitory concentration to acyclovir from 10 to 2.1 μM for HSV-1 and from 8 to 0.09 μM for HSV-2 (5). In dorsum-infected athymic nude mice and snout-infected hairless mice with infections due to wild-type HSV-1, wild-type HSV-2, and acyclovir-resistant strains of HSV-1 (TK deficient, TK altered, and DNA polymerase altered), the addition of topical 348U87 to topical acyclovir was shown to significantly decrease the area under the curve of the lesion score-day graph (15).

Since 1988, acyclovir-resistant HSV infections have been reported with increasing frequency in human immunodeficiency virus (HIV)-infected patients, and alternative therapies are needed (12). We evaluated a topical cream containing 348U87 (3%) and acyclovir (5%) in the treatment of acyclovir-resistant anogenital HSV infection that was unresponsive to high-dose systemic acyclovir therapy.

MATERIALS AND METHODS

HIV-infected patients with CD4 cell counts of less than 200/mm^3, a Karnofsky performance score of 80 or higher, and the presence of culture-proven cutaneous herpetic lesions that failed to respond to 10 days or more of therapy with either oral (≥30 mg/kg of body weight per day) or intravenous (≥5 mg/kg every 8 h) acyclovir were enrolled. Pretherapy virus isolates from all patients were documented

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TABLE 1. Study entry characteristics of patients treated with 348U87-acyclovir cream for acyclovir-resistant HSV-2 infections

Patient no.	Gender	CD4 cell count/mm^3	Location(s) of lesion(s)	Duration of lesion prior to entry (wks)	Acyclovir ID_{50} (μg/ml)
1	Male	40	Sacral area	7	19.2
2	Male	NA[a]	Perianal area	9	90.6
3	Male	103	Perianal area	16	40.6
4	Male	8	Perianal area	6	11.0
5	Male	40	Perianal area	3	61.3
6	Male	5	Perianal area	157	7.23
7	Male	15	Perianal, buttocks, and scrotum areas	10	16.0
8	Female	15	Perianal area	52	36.4
9	Male	9	Perianal area	26	3.1
10	Female	50	Perianal, vulvar, and buttocks areas	36	90.3

[a] NA, not available.

as acyclovir resistant (i.e., the inhibitory concentration of drug required to inhibit viral cytopathic effect by 50% [ID_{50}] was 3 μg/ml or higher), as performed at the Burroughs Wellcome Virology Laboratory by the neutral red dye uptake method (9). Those who required continued therapy with acyclovir, ganciclovir, or foscarnet were excluded from the trial. A negative pregnancy test was required for females prior to enrollment. Informed consent from each patient was obtained prior to initiation of study drug therapy, and the protocol and consent forms were approved by the Institutional Review Board at the University of California, San Francisco, and the University of Washington, Seattle.

The combination of 348U87 (3%) and acyclovir cream (5%) was applied topically to target (defined as the largest of the cutaneous herpetic lesions) and satellite lesions every 3 h for a maximum of six applications per day. Therapy was to be administered for a minimum of 14 days, at which time the study drug was discontinued if no reepithelialization was evident. Therapy was permitted to continue for a maximum of 42 days, until complete reepithelialization, failure to reepithelialize, or intolerance. Patients were seen on the 1st, 2nd, 5th or 6th, 9th, 10th, or 11th, and 14th or 15th days of therapy; once-weekly visits were required thereafter until discontinuation of the study medication. During each clinical evaluation, the area of the target lesion was measured (length by width), the level of pain was assessed by the patient on a scale from 0 to 3, the degree of erythema was assessed by the investigator on a scale from 0 to 3, and specimens were collected for virus culture by routine methods (2).

The primary efficacy variables were lesion size and duration of viral shedding; secondary efficacy variables included erythema scores and duration of pain. The factors predictive of healing were analyzed in two ways: by using two-by-three contingency tables of each predictor (e.g., size, duration, pretreatment acyclovir ID_{50}, and CD4 cell count) and outcome (complete, partial, or no response) and the chi-square test and by using the Spearman rank correlation coefficient to compare the numerical value of the predictor with that of the maximum percentage reduction achieved in the lesion during therapy.

RESULTS

Baseline characteristics. Ten HIV-infected patients (eight men and two women) with acyclovir-resistant anogenital HSV-2 infections were entered into the study (Table 1); median CD4 cell count was 15 cells per mm^3 (range, 5 to 103). The sizes of the target herpetic lesions at the time of study entry varied from 300 to 6,600 mm^2 (median, 1,050), and the durations of the lesions prior to entry varied from 3 to 157 weeks (median, 13). Previous therapy for the target lesions included oral acyclovir in all patients, intravenous acyclovir in four patients, intravenous foscarnet in five patients, and topical vidarabine (3% ophthalmic ointment) in two patients. A total of three patients had received treatment for acyclovir-resistant HSV prior to the episode for which they were enrolled in the study.

Clinical response. A total of 7 of 10 patients showed a decrease in target lesion area during the initial 14 days of therapy; the median size of the target lesion on day 14 was 433/mm^2 (range, 90 to 4,000). The seven patients who showed initial responses are classified as those whose lesions healed, those who had a plateau of response to the study drug, or those whose lesions relapsed while on therapy (Table 2). Only a single patient (no. 10) showed evidence of complete reepithelialization of target lesions during study drug therapy (Fig. 1a); however, a new vulvar lesion appearing on day 6 failed to heal, despite 37 additional days of study drug therapy. In three patients (nos. 1, 8, and 9), despite signs of early reepithelialization initially, the responses were felt to plateau in that the target lesion failed to decrease further in size despite continuation of therapy (Table 2; Fig. 1b). In three other patients (nos. 2, 4, and 7), relapse of the lesion (i.e., enlargement after initial reepithelialization) occurred while the patients were receiving study drug therapy (Fig. 1c). In patient no. 7, for example, the lesion healed completely after 9 days of therapy but enlarged thereafter, reaching one-third of its initial size by the completion of 34 days of therapy (Table 2). Similarly, in patient no. 2, the lesion had decreased in size by 17% on day 14 but enlarged to reach a size 13% greater than that at study entry, despite 2 weeks more of study drug therapy (Table 2).

Three patients (nos. 3, 5, and 6) failed to show any decrease in lesion size in response to study drug therapy (Table 2; Fig. 1d). However, in two patients, therapy was discontinued prior to day 14 because of causes unrelated to the study drug (death due to a pulmonary process in patient no. 6 on day 11 of therapy and intercurrent *Pneumocystis* pneumonia in patient no. 3 on day 10). Therapy was discontinued in patient no. 5 on day 7 because of burning on application and lack of apparent benefit.

An analysis of the factors predicting healing revealed that neither the size of the target lesion at the time of study entry, duration of the lesion, pretreatment acyclovir ID_{50}, nor CD4 cell count was associated with overall clinical response (P =

TABLE 2. Results of therapy with 348U87-acyclovir

Patient no.	Size of the target lesion (mm^2)		Smallest lesion size during therapy (mm^2) (% reduction)	Day of smallest lesion size	Total days of therapy	Category of response[a]	Development of new lesions (day of therapy)	Resolution of pain (day of therapy)
	At study entry	At completion of therapy (% change)						
1	1,500	1,275 (−15)	1,275 (15)	5	30	Plateau	Yes (9)	No
2	900	1,020 (+13)	750 (17)	14	28	Relapse	Yes (14)	No
3	400	500 (+25)	400 (0)	1	10	Failure	No	No
4	4,050	3,150 (−22)	2,420 (40)	6	11	Relapse	Yes (6)	No
5	300	300 (0)	300 (0)	1	7	Failure	No	No
6	1,500	1,500 (0)	1,500 (0)	1	11	Failure	No	No
7	300	100 (−67)	0 (100)	9	34	Relapse	No	Yes (6)
8	6,600	4,000 (−39)	3,600 (45)	11	40	Plateau	No	No
9	1,200	375 (−69)	375 (69)	40	40	Plateau	Yes (34)	No
10	325	0 (−100)	0 (100)	30	43	Healing	Yes (6)	Yes (24)

[a] Categories of response are for patients in whom complete reepithelialization of target lesion occurred (healing), in whom reepithelialization ceased to occur (plateau), in whom lesions improved and then worsened (relapse), and in whom no response was apparent (failure) during study drug therapy.

0.6, 0.4, 0.4, and 0.8, respectively, by chi-square analysis; $P = 0.8$, 0.5, 0.8, and 1.0, respectively, by the Spearman rank correlation coefficient).

Reduction in pain was observed for seven patients during therapy and sustained for five patients; for two patients, pain was eradicated. Reduction in erythema was noted for 8 of the 10 patients during study drug therapy; erythema remained decreased (6) or was absent (1) in the majority by the completion of therapy.

New lesions. A total of five patients (nos. 1, 2, 4, 9, and 10) developed new areas of ulceration adjacent to the target lesion during the study on days 9, 14, 6, 34, and 6 of therapy, respectively (Table 2). Virus cultures of the newly ulcerated areas demonstrated HSV for one patient and were negative

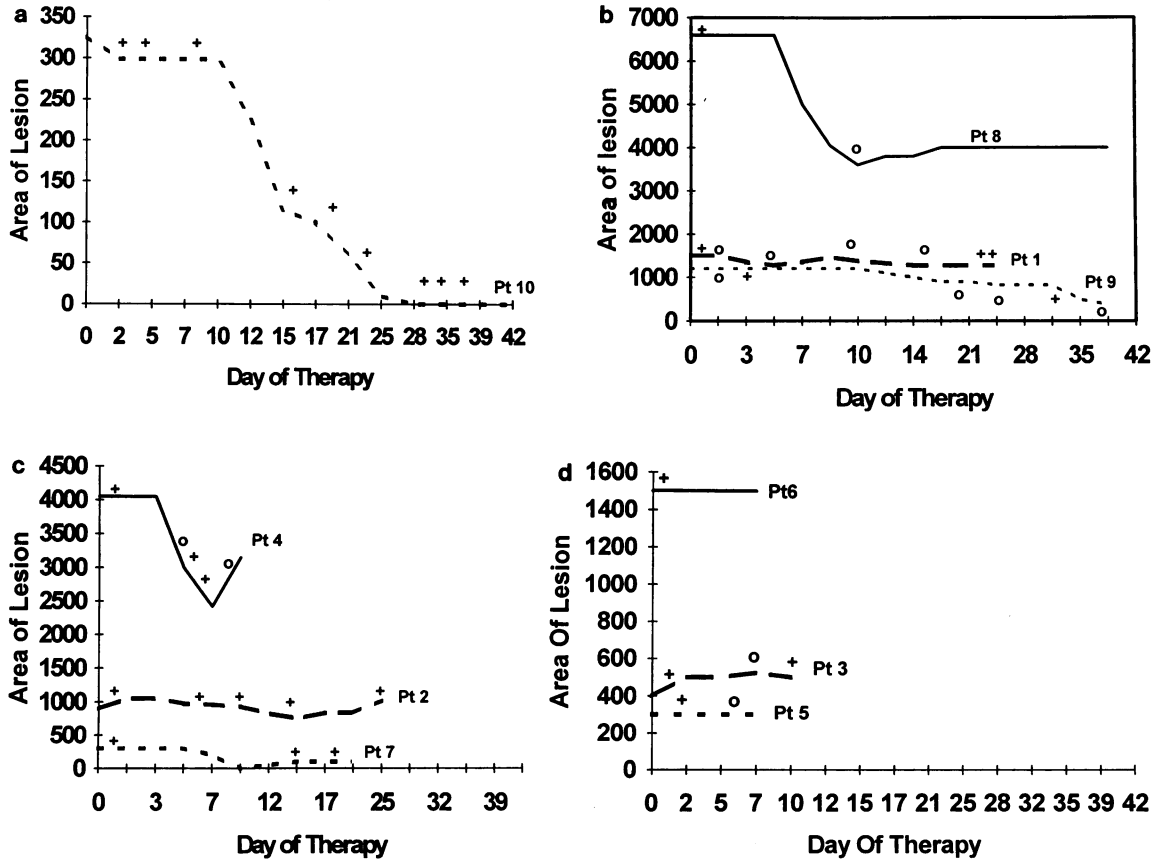

FIG. 1. Change in areas of the lesions and results of serial virus cultures for patients treated with 348U87 and acyclovir. (a) Patients who healed completely in response to study drug therapy; (b) patients in whom reepithelialization ceased to occur (plateau response); (c) patients in whom lesions improved and then worsened (relapsing response); (d) patients in whom no response was apparent during study drug therapy. Areas of the lesion are in square millimeters. +, recovery of HSV on virus culture; ○, a negative virus culture result.

for HSV for three patients; virus culture was not performed with one patient.

Virologic response. Serial virus cultures showed cessation of virus shedding in 6 of 11 patients; however, in 4 patients (nos. 1, 3, 4, and 9), cultures were transiently negative and became positive subsequently despite continuation of therapy (Fig. 1). In the single patient in whom target lesions healed completely (no. 10), virus cultures remained positive throughout therapy. In a total of three patients, virus cultures were persistently positive throughout therapy (nos. 2, 7, and 10).

Adverse effects. Four patients described a burning sensation when the study drug was applied (nos. 1, 3, 4, and 5). Marked erythema with ulceration (classified as new lesions [see above]) occurred in adjacent cutaneous areas in three patients (nos. 1, 2, and 4); given the negative virus culture results, we considered a dermatitic reaction as etiologic in these patients but were unable to confirm or exclude this possibility.

DISCUSSION

In this pilot study of 10 HIV-infected patients with mucocutaneous acyclovir-resistant anogenital HSV-2 infections, topical therapy with the combination of 348U87 and acyclovir yielded only a partial response. Although seven patients showed initial improvement, manifested by diminishment in the sizes of lesions and/or a decrease in symptomatology, complete healing was not achieved in any of our patients because of the apparent cessation of therapeutic effect after continued usage of the study drug, because of the emergence of new lesions in adjacent cutaneous areas which failed to respond to study drug therapy, or because of complaints of burning when the study drug was applied that resulted in premature discontinuation of therapy.

Lack of complete healing might be due to several factors. The advanced degree of immunosuppression in our patients (median CD4 cell count = 15/mm^3), in combination with the virustatic nature of acyclovir and 348U87, might result in impaired or absent healing despite substantial inhibition of virus replication. Of note is that five patients had received intravenous foscarnet as partial therapy before entry into the study; conceivably an alteration in the lesions or in the virus population due to foscarnet therapy may have occurred, such that response to the new agent was impaired (although a discrete explanation for why such a change might occur is lacking, a similar observation was made in a previous study of patients with mucocutaneous acyclovir-resistant HSV infections, such that patients who failed to heal in response to vidarabine therapy responded to subsequent therapy with foscarnet less well than those assigned initially to receive foscarnet [12]). Emergence of resistance to the ribonucleotide reductase inhibitor 348U87 during therapy is also a possibility; however, we were unable to test this hypothesis because of the insoluble nature of the compound in vitro, which made susceptibility testing difficult to perform. Conceivably, the high local concentrations of acyclovir afforded by topical administration were responsible for the observed partial therapeutic effect; the possibility that high concentrations of acyclovir are able to overcome resistant phenotypes in clinical lesions is suggested by a report of healing of cutaneous acyclovir-resistant HSV lesions in response to administration of acyclovir by continuous infusion (4). If this was in fact the case, then the development of incrementally higher degrees of acyclovir resistance during study drug therapy, resulting in cessation of the therapeutic effect apparent initially, is another theoretical possibility. Inadequate delivery of the study drug to the affected area by the topical route of administration is perhaps the most likely explanation for the observed lack of complete therapeutic effect; analogously, both topical acyclovir and topical foscarnet appear to be inferior in efficacy to systemic therapy in the treatment of recurrent genital herpes (8, 10, 11).

The lack of sustained clinical and virologic responses in our patients ultimately prompted the discontinuation of this trial; for the single patient in whom complete healing occurred, virus cultures were positive throughout therapy, and a new lesion appearing on day 6 enlarged progressively despite continuation of study drug therapy. Therefore, our results have not borne out the promise of the synergistic effect against acyclovir-resistant mutants seen in preclinical studies of combined 348U87 and acyclovir (5, 15). Future directions in the development of ribonucleotide reductase inhibitor compounds for the treatment of HSV infections must achieve either sufficient stability and safety to allow systemic administration or increased antiherpetic potency for topical use.

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