Antiviral Research, 23 (1994) 225–233

AVR 00684

Antiviral Research

Solar simulator-induced herpes simplex labialis: Use in evaluating treatment with acyclovir plus 348U87

D.I. Bernstein[a] and L.A. Rheins[b,1]

[a]*James N. Gamble Institute of Medical Research, 2141 Auburn Avenue, Cincinnati, Ohio 45219, USA and* [b]*Hilltop Research, Cincinnati, OH, USA*

(Received 28 September 1993; revised manuscript accepted 9 December 1993)

Summary

Models of UV radiation induced herpes labialis utilizing crude light sources have previously been used to examine the efficacy of antivirals. We sought to improve upon this model by using a solar simulator. Initial studies revealed that 13 of 34 (38%) subjects with a history of recurrent HSV labialis receiving three minimal erythema doses (MED's) of UV light developed herpes labialis. We next evaluated the effects of combined therapy with topical ACV and 348U87, a ribonucleotide reductase inhibitor, begun immediately after UV exposure for the prevention and treatment of herpes labialis. No significant reduction in the incidence or severity of herpes labialis was detected although the study was terminated after the interim analysis revealed no benefit, thus reducing the power to detect a difference. This lack of effect may be explained by the general poor efficacy of topical treatment for recurrent HSV infection. Further studies of ACV + 348U87 in vehicles that should increase the penetration and stability of the drugs are planned.

Herpes simplex labialis; Acyclovir; 348U87

Introduction

Recurrent herpes simplex labialis is a common infection affecting nearly one-third of the US population (Overall, 1984). There are an estimated 100 million

*Corresponding author. Fax: +1 (513) 241 3899.
[1]Present address: Advanced Tissue Sciences, 10933 N. Torrey Pines Road, La Jolle, California 92037-1005, USA.

SSDI 0166-3542(93)E0070-A

episodes of recurrent disease annually, yet the efficacy of existing antiviral therapy has been disappointing, especially with topical therapies (reviewed in Leigh, 1988; Raborn et al., 1988; Spruance and Freeman, 1990). In contrast, oral therapy with ACV has been more encouraging (Schadelin et al., 1988; Spruance et al., 1988; Rooney et al., 1993). For example, in the most recent study, Rooney et al. (1993) showed that daily ACV, significantly decreased recurrences by about 50%. Nevertheless, there are certain advantages to topical therapy that makes this a more desirable route for drug administration (Spruance and Freeman, 1990).

In recent studies, the addition of a ribonucleotide reductase inhibitor to topical ACV therapy proved to be synergistic in animal models of HSV disease (Ellis et al., 1989; Lobe et al., 1991; Spector et al., 1992), mimicking its in vitro effects (Spector et al., 1989). Ribonucleotide reductase is an enzyme that catalyzes the conversion of ribonucleotides to deoxyribonucleotides and is thus an important enzyme in the synthesis of viral DNA. Herpes simplex virus encodes a ribonucleoside that is distinct from its mammalian counterpart and thus could serve as a target for antiviral therapy. Experiments in mice indicate that the ribonucleotide reductase plays a role in pathogenesis but it is unclear if it is important in human HSV disease because although RR negative mutants grow poorly in mouse cells at 38°C, they replicate relatively efficiently in dividing human cells at 37°C (Cameron et al., 1988; Jacobson et al., 1989; Goldstein and Weller, 1988).

Ribonucleotide reductase inhibitors also potentiate the effect of ACV (Spector et al., 1989). This is believed to occur because ribonucleotide reductase inhibition prevents the increase in dGTP pools and increases the ACV-TP pool size thereby reducing the competition of dGTP for the binding of ACV-TP to the HSV DNA polymerase, the target enzyme (Spector et al., 1989, 1992). We chose to evaluate the RR inhibitor 348U87 because it is highly active and was not hematologically toxic (Spector et al., 1992). Thus it was of interest to determine if this combination of ACV + a ribonucleotide reductase inhibitor, 348U87 would be effective in prevention or treatment of herpes labialis.

A UV radiation-induced herpes labialis model has previously been used to examine the efficacy of oral and topical ACV preparations (Spruance et al., 1991). The results obtained with this model closely mimic studies done in naturally occurring HSV labialis. The light source used in these studies, however, were crude and we therefore sought to improve upon the model by using a solar simulator

Materials and Methods

Patient population

Healthy adults with a history of sunlight-induced herpes labialis and at least two episodes of herpes labialis in the preceding year were recruited. The protocols were approved by the Hilltop Research Institutional Review Board

committee on human studies. Signed informed consent was obtained from all subjects. All women had a negative pregnancy test and used adequate means of contraception during the trials. Use of antiinflammatory medication within 1 week, or immunomodulatory drugs, or antiviral mediation within 30 days was not permitted. Use of lip balm, cosmetics, soaps, fragrances, or medication known to produce abnormal response to sunlight were also prohibited

Light source

The ultraviolet light source used in this study was a Kratos solar simulator. This source consists of a 1000 watt xenon arc lamp mounted in a metal housing. The lamp's output is collected by an ellipsoidal mirror positioned just above the lamp and is reflected into a dichroic mirror. Much of the infrared and visible wavelength radiation passes through the mirrors, while the ultraviolet (UV) and some visible and infrared radiation are reflected. The beam of light emerges from the lamp housing horizontally, passing through a collimating lens, and onto a second dichroic mirror (mounted at a 45 degree angle) in a 90 degree light deflection tube. Again, much of the visible and infrared radiation is transmitted through the mirror while the UV and some visible and infrared radiation is reflected downward and passes through a 1 mm WG320 filter and a 2 mm UG-11 visible/infrared absorbance filter.

The resulting uniform (within $\pm 10\%$) beam of light is approximately four inches in diameter at a distance of about eighteen inches from the surface of the second dichroic mirror. This beam forms the optical output which is rich in UVB and UVA radiation. Some visible and infrared radiation is present as well; however, due to the dichroic mirrors, these wavelengths have been reduced to acceptable levels. The light source was positioned eighteen inches from the skin surface.

Energy measurements are recorded at the skin level using an optronic 742 spectrometer. Determinations of the uniformity of the field were made each day prior to the exposure of the subjects. Spectral output of the lamp was between 280–400 nm with the peak emission at 330 nm

Determination of minimal erythema dose (MED)

The MED was determined by exposing five 1 cm^2 areas of the volar forearm to sequentially increase doses of UV such that exposure to each area was 25% greater than the previous area. The test area was observed at 24 h and the MED defined as the minimum dose that produced the minimal perceptable erythema. The MED for subjects ranged from 60 to 190 s, with a mean of 104.3 ± 28.3 s.

Exposure of lips to UV light

A quadrant of the lips identified by the subject as the area of usual HSV recurrence was irradiated with the appropriate dose of UV within 5 days of determining the MED. A template with an 1×5 cm cutout was secured in place over the appropriate perioral quadrant. Nontargeted sites were protected with SPF 15 sunscreen and then appropriately draped.

Development of model

Initial studies were performed on forty subjects, six receiving 4 MEDs and thirty-four receiving 3 MEDs to establish the utility of using the solar simulator to induce HSV recurrences. Following UV exposure of the lips, all subjects were examined on days 1 and 3 by the investigator. Subjects were also instructed to notify the investigator and report to the study site within 24 h of developing any lesion compatible with an HSV recurrence. All subjects also kept a diary and were contacted on day 5, 7, and 9 to insure compliance. All subjects developing lesions were seen daily for 5 days and then every other day until complete resolution of the lesions. The approximate time of lesion development was obtained by interview of the patient and was defined as the subject's first awareness of a papule or induration. Clinical assessment of lesion severity was made by observation of lesion stage and pain. All lesions developing within 1 cm of the exposed area and within 7 days during the follow up period were included in the analysis.

Drug evaluation

A subsequent double blind evaluation was performed approximately 6–17 months later on 51 subjects to assess the effects of a combination of topical acyclovir and 348U87 compared to placebo on lesion development and severity. Subjects were randomly assigned according to a code supplied by the sponsor. UV radiation exposure was performed as described above. Immediately after UV exposure, subjects began treatment by application of the study medication to the UV exposed quadrant. The cream was applied every 2 h, while awake (maximum 8 applications/day) for 7 days. If lesions developed, treatment was continued until the lesions healed up to maximum of 5 additional days.

The formulation cream consisted of 5% acyclovir and 3% 348U87 in a 40% propylene glycol base or a placebo cream supplied by Burroughs Wellcome Research, Triangle Park, North Carolina.

Virus isolation

A swab specimen was obtained twice from suspected lesions after rupturing vesicles on days 1–3 after lesion appearance and placed in transport medium. A 0.2 ml aliquot was then inoculated immediately onto Vero cells which were observed for 7 days for CPE typical of HSV.

Statistical analyses

The sample size for the drug evaluation was estimated to be fifty patients per group to achieve a power of 80% and a significance level of 0.05 if the drug decreased recurrences by 60%. An interim analysis after fifty subjects was planned.

Results

Development of model

Initially six patients were enrolled and received a 4 MED exposure to one quadrant of the lip. Two of these patients developed lesions consistent with herpes labialis and were HSV culture positive. However, an additional three subjects had lesions compatable with herpes labialis that were culture negative and felt most likely to be reactions to the UV exposure. Therefore, the remaining thirty-four subjects received 3 MED exposure. As shown in Table 1,

TABLE 1

Development of perioral herpes lesions* following UV-exposure

MED	N	Subjects developing HSV(+) lesions	Number HSV(+) lesions	Subjects developing any lesion	Number of lesions
4	6	2 (33%)	4	5 (83%)	7
3	34	13 (38%)	17	15 (44%)	20

*Only lesions consistent with herpes labialis are shown.

thirteen (38%) of the thirty-four subjects developed seventeen HSV culture (+) lesions. An additional three lesions from two subjects were felt to be compatible with herpes labialis but not characteristic. All were culture negative. Thus

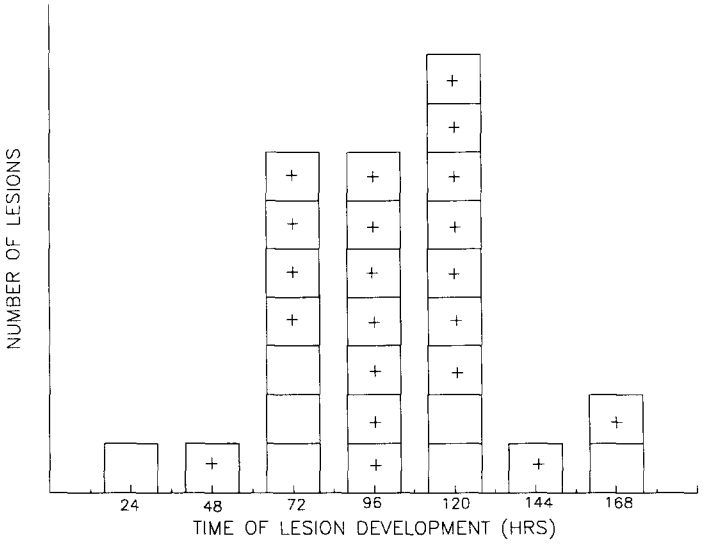

Fig. 1. Induction of perioral lesions by solar simulator. Subjects were exposed to 3–4 MED of UV light and the onset of lesions compatible with herpes labialis within the exposed area recorded. Lesions with HSV isolated are marked by a (+).

TABLE 2(a)

Effect of treatment on development of perioral herpes lesions

Treatment	N	Subjects developing HSV(+) lesions	Number HSV (+) lesions	Subjects developing any lesion	Number of lesions
Placebo	26	4 (15.4%)	5	5 (19.2%)	6
ACV + 348U87	25	3* (12.0%)	4	7 (28.0%)	9

*One other subject developed HSV(+) lesions outside the irradiated area.

TABLE 2(b)

Effect of treatment on HSV(+) lesions

Treatment	N	Mean days to hard crust	Mean days to heal	Maximum lesion size (cm^2)
Placebo	5	2.3 ± 1.0	6.8 ± 1.3	70 ± 40
ACV + 348U87	4	2.7 ± 0.6	9.3 ± 3.8	143 ± 112

reduction from 4 to 3 MEDs reduced the reactions caused by UV exposure without decreasing the reactivation of HSV.

The distributions over time for the development of lesions consistent with herpes labialis after 3 or 4 MED exposure is shown in Fig. 1. All but two lesions [one HSV(+)] were observed at or after 48 h post-UV exposure. Most lesions (81%) including 86% of HSV(+) lesions were observed between 72 and 120 h

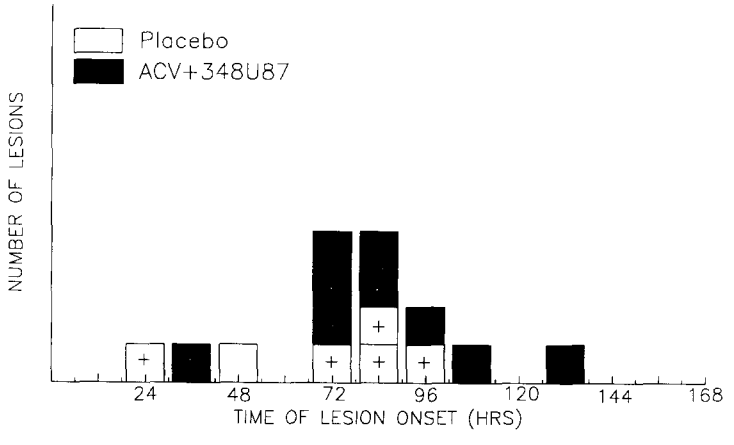

Fig. 2. Effect of therapy with ACV + 348U87 on induction of perioral lesions. Subjects were exposed to 3 MED of UV light and treated immediately with ACV + 348U87 or placebo. The onset of lesions compatible with herpes labialis within the exposed area was recorded. Lesions with HSV isolated are marked by a (+).

after UV exposure.

Evaluation of ACV + 348U87 cream

As shown in Table 2a, there was no significant difference in the number of subjects developing HSV culture positive or any lesions consistent with herpes labialis comparing placebo recipients to subjects treated with topical ACV plus 348U87. Similarly there was no difference in the number of lesions that developed comparing placebo and drug recipients. There was also no evidence that treatment reduced the severity of the HSV culture positive lesions that developed either by comparing the maximal size or time to healing or crusting (Table 2b). In fact for all evaluations, the treated group responded less well although the differences were not significant ($P > 0.2$). Similarly, when comparisons were made using all lesions, the severity for all the parameters evaluated were equivalent ($P > 0.5$). Because there was no trend for the benefit of the drug treatment the study was discontinued after the interim analyses.

The distribution over time for the development of lesions is shown in Fig. 2. Only three lesions developed before 48 h after UV exposure.

Discussion

In these studies we found that induction of perioral HSV lesions using the solar simulator was similar but no better than previous studies which utilized a crude source of UV light (Spruance, 1991). Reduction of exposure from 4 MEDs to 3 MEDs reduced the induction of lesions that were felt to be the direct effect of UV exposure that has also been noted in other evaluations (Spruance et al., 1991).

The development of early HSV lesions (<48 h after UV exposure) has been noted in some investigations (Spruance et al., 1991; Rooney et al., 1991) but not by others studying UV reactivation of HSV-2 (Rooney et al., 1992). In our smaller study we detected the appearance of lesions before 48 h in three of 42 cases (7%). The large majority of lesions appeared at a time consistent with the time it would take for virus to be reactivated from the site of latency in the trigeminal ganglia, be transported to the labial epidermis by neural transport, and replicate to produce a typical lesion.

Using this model we were able to evaluate the combination of topical ACV + 348U87 for prevention and treatment of UV-induced HSV labialis. Unexpectedly, the rate of induction decreased from the 38% observed in our trials to establish the model to 14%. An unlikely explanation for this would be that the vehicle inhibited HSV lesion development. Nevertheless, it appeared that therapy was ineffective as there was no difference or even apparent trends in reducing the development of herpes labialis or its severity. Because of the lack of apparent effects, the study was discontinued after the scheduled interim analysis decreasing the power to detect a significant difference. Similar disappointing results were also seen in a study using this same combination

therapy in ten patients with human immune deficiency virus and ACV resistent anogenital HSV infections (Safrin et al., 1993). In this study, transient improvement occurred frequently but lesions reepithelialized completely in only one of ten patients and treatment was terminated in most cases because of the cessation of therapeutic effects.

The lack of effect is probably best explained by the generally poor efficacy of topical treatment for recurrent HSV infections that is thought to result from the inadequate delivery of the drug to the affected area (reviewed in Spruance and Freeman, 1990) or to this specific combination of antivirals. In previous animal studies, evaluating this or similar combinations, the addition of a ribonucleotide reductase inhibitor was synergistic with ACV and provided effective therapy (Ellis et al., 1982; Luke et al., 1991). These studies, however, employed models where the skin is abraded prior to inoculation of HSV which could also increase the penetration of drugs. Further these models did not evaluate the effects on recurrent disease where lesions develop as virus emerges from neural endings. Even the studies of zosterform lesions (Lobe et al., 1991) were performed by treating the inoculated area and not the area of expected spread. Thus, it is possible that the impressive results observed in animals will not be observed in human trials. Nevertheless, further studies of ACV + 348U87 in vehicles that should increase the penetration and stability of the drugs are planned.

Acknowledgement

This work was supported by Burroughs Wellcome Company.

References

Cameron, J.M., McDougall, I., Marsden, H.S., Preston, V.G., Ryan, D.M. and Subak-Sharpe, J.H. (1988) Ribonucleotide reductase encoded by herpes simplex virus is a determinant of the pathogenicity of the virus in mice and a valid antiviral target. J. Gen. Virol. 69, 2607–2612.

Ellis, M.N., Lobe, D.C. and Spector T. (1989) Synergistic therapy by acyclovir and A1110U for mice orofacially infected with herpes simplex viruses. Antimicrob. Agents Chemother. 33, 1691–1696.

Goldstein, D.J. and Weller, S.K. (1988) Herpes simplex virus type 1-induced ribonucleotide reductase activity is dispensable for virus growth and DNA synthesis: isolation and characterization of an ICP6 *lac*Z insertion mutant. J. Virol. 62, 196–205.

Jacobson, J.G., Leib, D.A., Goldstein, D.J., Bogard, C.L., Schaffer, P.A., Weller, S.K. and Coen, D.M. (1989) A herpes simplex virus ribonucleotide reductase deletion mutant is defective for productive acute and reactivatable latent infections of mice and for replication in mouse cells. Virology 173, 276–283.

Leigh, I.M. (1988) Management of non-genital herpes simplex virus infections in immunocompetent patients. Am. J. Med. 85 (Suppl. 2A), 34–38.

Lobe, D.C., Spector, T. and Ellis, M.N. (1991) Synergistic topical therapy by acyclovir and A1110U for herpes simplex virus-induced zosteriform rash in mice. Antiviral Res. 15, 87–100.

Overall, J.C., Jr. (1984) In: Galasso, G.J., Merigan, T.C. and Buchanan, R.A (Eds), Antiviral

Agents and Viral Diseases of Man (2nd Ed), pp. 251–252. Dermatologic viral diseases. New York, Raven Press.

Raborn, G.W., McGaw, W.T., Grace, M. and Percy, J. (1988) Treatment of herpes labialis with acyclovir: review of three clinical trials. Am. J. Med. 85 (Suppl. 2A), 39–42.

Rooney, J.F., Bryson, Y., Mannix, M.I., Dillon, M., Wohlenberg, C.R., Banks, S., Wallington, C.J., Notkins, A.L. and Straus, S.E. (1991) Prevention of ultraviolet-light-induced herpes labialis by sunscreen. The Lancet 338, 1419–1422.

Rooney, J.F., Straus, S.E., Mannix, M.L, Wohlenberg, C.R., Alling, D.W., Dumois, J.A. and Notkins, A.L. (1993) Oral acyclovir to suppress frequently recurrent herpes labialis: a double-blind, placebo-controlled trial. Ann. Int. Med. 118, 268–272.

Rooney, J.F., Straus, S.E., Mannix, M.L., Wohlenberg, C.R., Banks, S., Jagannath, S., Brauer, J.E. and Notkins, A.L. (1992) UV light-induced reactivation of herpes simplex virus type 2 and prevention by acyclovir. J. Infect. Dis. 166, 500–506.

Safrin, S., Schacker, T., Delehanty, J., Hill, E. and Corey, L. (1993) Topical treatment of infection with acyclovir-resistant mucocutaneous herpes simplex virus with the ribonucleotide reductase inhibitor 348U87 in combination with acyclovir. Antimicrob. Agents Chemother. 37, 975–979.

Schadelin, J., Schilt, H.U. and Rohner, M. (1988) Preventative therapy of herpes labialis associated with trigeminal surgery. Am. J. Med. 85 (Suppl. 2A), 46–48.

Spector, T., Harrington, J.A., Morrison, R.W., Lambe, C.U., Nelson, D.J., Averett, D.R., Biron, K. and Furman, P.A. (1989) 2-Acetylpyridine 5-[(dimethylamino)thiocarbonyl]thiocarbono hydrazone (A111OU), a potent inactivator of ribonucleotide reductase of herpes simplex and varicella-zoster viruses and a potentiator of acyclovir. Proc. Natl. Acad. Sci. USA 86, 1051–1055.

Spector, T., Lobe, S.C., Ellis, M.N., Blumenkopf, T.A. and Szczech, G.M. (1992) Inactivators of herpes simplex virus ribonucleotide reductase: hematological profiles and in vivo potentiation of the antiviral activity of acyclovir. Antimicrob. Agents Chemother. 36, 934–937.

Spruance, S.L. and Freeman, D.J. (1990) Topical treatment of cutaneous herpes simplex virus infections. Antiviral Res. 14, 305–321.

Spruance, S.L., Freeman, D.J., Stewart, J.C.B., McKeough, M.B., Wenerstrom, L.G., Krueger, G.G., Piepkorn, M.W., Stroop, W.G. and Rowe, N.H. (1991) The natural history of ultraviolet radiation-induced herpes simplex labialis and response to therapy with peroral and topical formulations of acyclovir. J. Infect. Dis. 163, 728–734.

Spruance, S.L., Hamill, M.L., Hoge, W.S., Davis, L.G. and Mills, J. (1988) Acyclovir prevents reactivation of herpes simplex labialis in skiers. J. Am. Med. Assoc. 260, 1597–1599.