The Natural History of Ultraviolet Radiation–Induced Herpes Simplex Labialis and Response to Therapy with Peroral and Topical Formulations of Acyclovir

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The lips of 196 patients with a history of sun-induced herpes labialis were exposed to experimental ultraviolet radiation (UVR) and treated with acyclovir (ACV) or placebo at different times and by different routes. Of 98 placebo recipients, 39 (40%) developed 43 lesions inside or within 10 mm of the irradiated zone. The temporal distribution of lesions was bimodal, 11 (26%) occurring within 48 h (immediate) and 32 (72%) 2-7 days after UVR exposure (delayed). Prophylactic peroral ACV begun 7 days before or 5 min after UVR prevented the development of the delayed but not the immediate lesions (P < .001). When peroral ACV was started 48 h after UVR, delayed lesions developed but were less severe (P = .01-.05). Prophylactic topical ACV begun 5 min after UVR did not reduce lesion frequency or severity. ACV therapy can be efficacious, but some rapidly developing lesions are unresponsive to treatment. This suggests that more than one process may contribute to the pathogenesis of herpes labialis.

There are widely differing views in the literature concerning the efficacy of prophylactic or therapeutically administered acyclovir (ACV) for the management of recurrent herpes simplex labialis in immunocompetent hosts. Prophylactic ACV cream was used successfully by one investigator to prevent herpes labialis [1] but failed in another trial [2]. When used for the treatment of a developing lesion, topical ACV cream was associated with an increased frequency of aborted lesions [3], whereas other trials of ACV cream or ACV capsules for the treatment of herpes labialis noted a decrease in lesion severity without a change in the number of aborted lesions [4-6] or no effect at all [7, 8]. Neither topical nor peroral ACV is approved in the United States for herpes labialis in immunocompetent hosts, although both are prescribed extensively by physicians and dentists for this disease and are often described anecdotally to be of value (unpublished data).

When ACV or placebo capsules were administered prophylactically to skiers in a study of sun-induced herpes labialis, the frequency of herpes labialis was similar among the two treatment groups during the first 4 days of the study,[9]. On study days 5-7, a large number of cases developed among placebo recipients but none among those receiving ACV. While the overall reduction in lesion frequency by ACV was impressive (73%) and highly significant (P = .001), the failure of prophylactic drug therapy to prevent lesions early in the study was without a ready explanation.

To explain these mixed observations and to learn how best to administer antiviral agents to patients with herpes labialis, we developed an experimental model system in which ultraviolet radiation (UVR) induces herpes labialis in human subjects [10]. We used this model to study the prophylactic and therapeutic efficacy of topical and peroral formulations of ACV and the influence on efficacy of different times of initiation of treatment.

Materials and Methods

Patient population. Three consecutive studies of peroral ACV were conducted at the University of Utah School of Medicine; in addition, a multicenter study of topical ACV was conducted at the University of Utah School of Medicine, the University of Michigan School of Dentistry, and the Graduate School of Public Health, University of Pittsburgh (table I). The patients were recruited from among individuals with a typical clinical history of recurrent herpes labialis: episodes of vesicular lesions on the vermilion border of the lips or on the perioral skin. In addition, the etiology of the lesions was documented in every instance by prior isolation of herpes simplex virus (HSV) from lesion samples. All patients had a history of reactivation of herpes labialis by exposure to sunlight and had a history of lesions usually occurring on one specific area of the lips. All participants were ≥18 years old and in good general
The proportion of patients who had used an antiviral medication in the preceding 4 weeks were excluded. All women had a negative urine pregnancy test and used adequate means of contraception during the trial period.

**Study medication.** Five percent ACV cream and placebo cream were provided in identically appearing 15-g tubes by Burroughs Wellcome (Research Triangle Park, NC). The cream vehicle used in this study differed by the absence of sodium lauryl sulfate from the cream vehicle licensed in Europe and previously studied in our laboratory [11, 12]. Topical ACV in the new cream was less irritating to the skin yet maintained activity comparable to that of the older cream preparation and greater than that of the ACV ointment in the dorsal cutaneous guinea pig model of HSV-1 infection (Collins P, Wellcome Research Laboratories, personal communication). Gelatin capsules (Eli Lilly, Indianapolis) were filled with 200 mg of ACV from commercially available capsules (Burroughs Wellcome) or lactose placebo compound and randomly allocated to serially numbered bottles. The drug code for topical and peroral clinical trial materials was concealed from both patients and investigators until the end of the study. Drug dosing is described in Results.

**Determination of the minimal erythema dose (MED).** Six small circular, 1-cm-diameter areas of each patient’s ventral forearm were exposed to UVR from two fluorescent tubes (FS20; Westinghouse, Bloomfield, NJ) at a distance of 19 em for periods of 2, 3, 4, 5, 6, or 7 min (17-58 J/cm²). This radiation source emits 30% of its energy as UVA (400-320 nm), 62% as UVB (320-290 nm), and 8% as UVC (290-200 nm) (product specifications). The shortest duration of exposure producing a homogeneous erythematous reaction with distinct margins in 24 h was defined as 1 MED.

**Exposure of lips to UVR.** A quadrant of the lips that was the usual site of recurrent herpes labialis for each individual patient was outlined with a black marking pen. The demarcated zone began in the midline on the perioral skin 0.5-1 cm from the vermilion border and extended across to the corner of the mouth and back to the midline along the junction of the vermilion border with the oral mucous membrane (an area ~3 cm²). The rest of the lips and perioral skin outside this zone was covered with a paminobenzoic acid sunscreen having a sun protection factor of 15. The fluorescent tubes were rotated to face the patients and an opaque shield with an oval hole was positioned 19 em from the tubes, the same distance and intensity of exposure used to determine the MED on the arm. The patient positioned his or her lips in the opening and was irradiated for 3-4 MED by increasing the duration of exposure.

**Reactions to UVR exposure included pain or tingling of mild-to-moderate intensity and, in some patients, a thin rim of erythema corresponding to the irradiated area of perioral skin. These effects were most pronounced 24 h after UVR exposure and were typical of a “sunburn.” A few patients developed watery, painless, nonindurated blisters without an antecedent papule stage or evolution to an ulcer.** Viral cultures of fluid from these blisters were uniformly negative. Tissue from biopsies in two cases were also viral culture-negative, and examination by light microscopy revealed mild inflammatory changes without HSV cytopathology. These blisters were considered a direct effect of UVR exposure and not a manifestation of HSV reactivation.

**Evaluation of lesions.** Patients were studied every other day for four visits for evidence of herpes labialis. The exact time of lesion onset was obtained historically by patient interview and was defined as the patient’s first awareness of a papule or induration. Clinical assessment of lesion severity was made by observation of lesion stage, size, and pain by previously described criteria [6]. Lesion specimens for isolation of virus were taken during the first 3 lesion days by scraping the surface of papules, breaking vesicles, and absorbing vesicle fluid into a cotton swab and swabbing the base of ulcers. Swabs were placed into 2 ml of viral transport media and processed for viral isolation as described below.

Some immediate lesions were further assessed by a 2-mm punch biopsy. Local anesthesia was achieved by injection of 1% lidocaine. Half the specimen was placed in viral transport medium and assayed for infectious virus by ultracentrifugal inoculation of tissue culture monolayers (see below). The other half was exposed first to PLP fixative (1 mM sodium m-periodate, 75 mM lysine, 2% paraformaldehyde in 37 mM phosphate buffer, pH 7.4) and subsequently to 70% ethanol and 30% water, thin sectioned, stained with hematoxylin and eosin, and examined for cell morphology by routine light microscopy.

**Viral isolation.** Lesion swab and scrape specimens in viral transport medium were plated onto monolayers of susceptible cells. The presence of HSV in the specimen was determined by the appearance of typical cytopathic effects. Lesion biopsy specimens were minced with a scalpel in transport medium and used to inoculate mink lung cell monolayers in 60-mm six-well plates (Linbro Scientific, Hamden, CT). The plates were covered with sterile adhesive wrapping, centrifuged at 1400 g for 60 min at 4°C, and then incubated at 37°C for 7 days and observed for the appearance of HSV cytopathic effects. Some isolates were further identified as HSV types 1 or 2 by fluorescein-conjugated monoclonal antibody procedure (Syva, Palo Alto, CA).

**Sensitivity of viral isolates to ACV.** The in vitro sensitivity of selected viral isolates to ACV by the dye uptake method [13] was done by Nixon Ellis (Burroughs Wellcome).

**Data analysis.** The proportion of patients in each treatment group that developed lesions within a 7-day period after UVR exposure was compared by Fisher’s exact test or by χ² analysis. Other measures of lesion severity were examined by the Mann-Whitney rank sum test. All probability determinations were two-tailed and P < .05 was considered significant.

**Table 1. Therapeutic trials of acyclovir (ACV) in ultraviolet radiation–induced herpes labialis.**

<table>
<thead>
<tr>
<th>Experiment, route of administration</th>
<th>Day treatment began*</th>
<th>Length of treatment (days)</th>
<th>No. patients treated with ACV</th>
<th>No. patients treated with Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroral</td>
<td>0</td>
<td>7</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Peroral</td>
<td>-7</td>
<td>14</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Early treatment.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroral</td>
<td>2</td>
<td>5</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Prophylaxis Topical</td>
<td>0</td>
<td>7</td>
<td>45</td>
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* Day 0 was the time of ultraviolet radiation exposure; -7 indicates treatment was started 7 days before radiation exposure.

**Results**

**Induction of herpes labialis by exposure to experimental UVR.** A total of 196 patients were exposed on the lips to...
experimental UVR to induce a recurrence of herpes labialis and treated with 5% ACV cream, cream control, ACV capsules, or placebo capsules beginning at different times as described below (table 1). The volunteers were mostly Caucasian (98%) and female (71%) and ranged in age from 18 to 66 years (mean, 33). By history, the mean frequency of herpes labialis in the study group under natural circumstances was 5.2 episodes per year. Of the volunteers, 59% considered themselves to be at “high risk” of a recurrence after sun exposure (developing lesions more than half of the time) and 41% were “low risk” (less than half the time).

To characterize the natural history of experimental UVR-induced herpes labialis, we separately examined the data from the 98 subjects exposed to UVR and treated with peroral or topical placebo formulations. Thirty-nine (40%) of the 98 subjects developed 43 separate lesions or lesion clusters within 7 days after UVR exposure; these were inside the UVR-exposed zone (39 lesions) or within 10 mm of the zone (4 lesions). Three additional lesions occurred distant from the UVR-exposed zone; because of the infrequency of such lesions, the inability to treat them prophylactically with topical ACV, and their uncertain relationship to UVR exposure, they were not further considered in this analysis.

The development of UVR-induced lesions in placebo recipients as a function of the time interval between UVR exposure and lesion onset is shown in figure 1. The distribution of the 43 lesions was bimodal. 11 (26%) occurring within 48 h with a peak at 12–24 h (immediate lesions) and 32 (74%) developing 2–7 days after the stimulus with a peak at 72 h (delayed lesions). Patients developed immediate or delayed lesions but not both. The development of a lesion in this model did not correlate with age, sex, prior natural frequency of herpes labialis, or self-perceived risk from sun exposure.

The clinical severity of immediate and delayed lesions was compared. The immediate lesions were less severe by a variety of measures including frequency of aborted lesions, maximum size, duration of pain, healing time, and frequency of positive viral cultures; but only the difference in frequency of positive cultures was statistically significant (5/11 vs. 28/32, 1 \( P < .01 \)). Immediate lesions also had a tendency toward a longer mean (median) duration of the papule stage, 1.1 (0.7) versus 0.3 (0.1) days, 1 \( P < .08 \), and a shorter mean (median) duration of the hard crust stage, 3.6 (3.6) versus 6.1 (5.9) days, 1 \( P = .08 \). Virus was recovered from one or more patients with immediate lesions at each of the three study centers. Two patients with immediate lesions were studied by lesion biopsy. HSV was isolated from both, and typical HSV histopathology of the epidermis was seen. Four isolates from immediate lesions and 10 from delayed lesions were typed: All were HSV type 1.

**Prophylactic peroral treatment of UVR-induced herpes labialis with ACV capsules or placebo.** Thirty patients were treated for 7 days with ACV capsules (200 mg, five times/day) or placebo capsules, beginning immediately after UVR exposure. Another 36 patients received peroral drug or placebo for 14 days beginning 7 days before UVR exposure (table 1). Because the results with the two dosing schemes were the same, the data were combined. The demographic characteristics of the patients were similar to those of the total population (see above), and there were no significant differences between treatment groups (data not shown).

As shown in figure 2, 6 (18%) of 33 patients treated with ACV capsules developed a total of six lesions compared with 13 (39%) of 33 patients with 13 lesions among the placebo capsule recipients (1 \( P = .10 \)). The effect of peroral ACV was profound on the delayed lesion subgroup, where the development of lesions was completely inhibited (1 \( P < .001 \)). Prophylactic peroral ACV therapy had no effect on the development of immediate lesions, whether begun at the time of UVR exposure (three immediate lesions, two culture positive) or 7 days in advance (three immediate lesions, one culture positive).

**Effect of peroral ACV treatment begun 48 h after UVR.** To further examine the relationship between efficacy and time of initiation of drug prophylaxis and to identify a dosing regimen in the model that would simulate early treatment, 40 patients were treated with ACV or placebo capsules (200 mg five times/day) for 5 days beginning 48 h after UVR exposure, immediately before the usual time of onset of delayed lesions. The demographic characteristics of the patients were similar to those of the total population (see above), and there were no significant differences between treatment groups (data not shown). In the following analysis, only lesions developing after the start of therapy (the delayed lesions) were studied.

![Figure 1. Distribution of herpes labialis lesions by time of lesion onset among 98 patients exposed to ultraviolet radiation (UVR) on the lips and treated with placebo formulations. Lesion onset was defined as the beginning of the papule stage. +, viral culture-positive lesion.](image-url)
Figure 2. Effect of prophylactic peroral treatment with acyclovir (A) or placebo capsules (B) on development of herpes labialis lesions among 40 patients exposed to ultraviolet radiation (UVR) on the lips. Treatment (200 mg, 5 times/day) was initiated 7 days before UVR exposure for 14 days (Rx1) or immediately after UVR exposure for 7 days (Rx2). Lesions are shown by time of onset, defined as the beginning of the papule stage. +, viral culture–positive lesion.

As shown in figure 3, 7 (35%) of 20 patients treated with ACV capsules developed seven delayed lesions compared with 8 (40%) of 20 patients with eight delayed lesions among the placebo capsule recipients \( (P = \text{NS}) \). Comparison of severity among the ACV- and placebo-treated lesions, respectively, revealed evidence of a marked benefit from drug therapy by several lesion measures: the mean (median) maximum lesion area, 52 (35) versus 153 (160) mm\(^2\), \( P < .01 \); the mean (median) healing time to loss of crust, 6.0 (5.8) versus 11.6 (11.8) days \( P < .05 \); and the mean (median) healing time to normal skin, 8.1 (6.0) versus 12.5 (12.9) days, \( P < .05 \). The frequency of lesions not progressing beyond the papule stage (aborted lesions), one of seven versus none of eight, \( P = .94 \); the mean (median) duration of pain 5.0 (2.0) versus 3.9 (4.8) days, \( P > .10 \); and the frequency of positive viral cultures, three of seven versus six of eight, \( P = .31 \), were not significantly different. The time interval between start of ACV treatment and lesion onset (papule stage) ranged from 2 to 34 for the seven lesions in this treatment subgroup; there was no correlation between the duration of this interval and the degree of reduction in lesion severity (data not shown).

Prophylactic topical treatment of UVR-induced herpes labialis with 5% ACV cream or cream vehicle control. Ninety patients were randomized to apply 5% ACV cream or cream control to the UVR zone every 2 h, while awake, for 7 days beginning immediately after UVR exposure. The demographic characteristics of the patients were similar to those of the total population (see above), and there were no significant differences between treatment groups (data not shown). There were no local or systemic adverse reactions to the test treatment.

As shown in figure 4, 22 (49%) of 45 patients treated with ACV cream developed a total of 31 lesions compared with 18 (40%) of 45 patients with 21 lesions among the vehicle control recipients. Neither immediate or delayed lesions were reduced in frequency by prophylactic treatment with ACV cream. Similar results were obtained at each of three study centers (data not shown).

Examination of lesion severity failed to reveal any significant differences between treatment groups. The values of five clinical measures of lesion severity were, for drug and placebo recipients, respectively: frequency of lesions not progressing beyond the papule stage (aborted lesions), 3 of 31 versus 2 of 21, \( P = 1.0 \); mean (median) maximum lesion area, 110 (36) versus 72 (56) mm\(^2\), \( P = .88 \); mean (median) duration of pain, 3.7 (3.5) versus 3.6 (3.8) days, \( P > .10 \); mean (median) healing time to loss of crust, 6.7 (7.0) versus 6.5 (7.1) days, \( P = .79 \); and mean (median) healing time to normal skin, 6.8 (7.4) versus 7.4 (7.3) days, \( P = .70 \). The frequency of positive viral cultures in the delayed lesion subgroup was significantly less for ACV than vehicle control recipients, 14 (58%) of 24 versus 11 (92%) of 12, \( P = .05 \), but there was no significant difference by treatment group for immediate lesions or for the total population.

In vitro sensitivity of HSV isolates to ACV. Thirteen HSV isolates were examined for sensitivity to ACV by the dye uptake procedure. Isolates from four immediate lesions ranged
in sensitivity (ED\textsubscript{50}) from 0.6–3.5 \( \mu \)g/ml (mean 1.4 \( \mu \)g/ml). Of nine isolates from delayed lesions, one from a patient treated with ACV cream was resistant (17.8 \( \mu \)g/ml); the other eight isolates were considered sensitive (range 0.6–1.9, mean 1.2 \( \mu \)g/ml).

**Discussion**

A total of 196 volunteers participated in a placebo-controlled, double-blind, multicenter study of ACV in the treatment of experimental UVR-induced herpes labialis. To understand the natural history of the experimental lesions, we examined the 98 placebo-treated subjects and found that 39 (40%) developed lesions within 7 days of irradiation of the lips. The induced lesions were clinically typical of recurrent herpes labialis in terms of lesion size, duration, stages, rate of viral isolation, HSV type, and HSV antiviral drug sensitivity [6, 9]. We made the novel observation that the UVR-induced lesions developed in a bimodal pattern by time after stimulus: 26% occurred within 48 h and the rest within 2–7 days.

We identified the bimodal pattern of lesion development because we stimulated HSV reactivation experimentally at a brief, fixed point in time and then carefully documented the time of lesion onset. The immediate lesion group was of particular interest because of the resistance of these lesions to prophylactic peroral ACV. In a survey of 106 patients with sunlight-induced herpes labialis, 22% of lesions began within 24 h of sun exposure (unpublished data), and among sunlight-exposed skiers, lesions appearing in the first 4 days after arrival at a resort were not prevented by prophylactic peroral ACV [9]. Douglas et al. [14] found that peroral ACV for the prophylaxis of recurrent herpes genitalis was not effective until after the first week of treatment. In contrast to these experiences, immediate lesions or a bimodal distribution of induced lesions have not been seen after UVR exposure of the buttocks, thighs, or penis [15–17] or after central stimuli such as trigeminal or thoracolumbar nerve surgery [18–20].

The pathogenesis of immediate lesions may differ from that of delayed lesions. HSV replication in the trigeminal ganglion in response to UVR, viral axonal transit, and seeding of the labial epidermis likely require several days to evolve [21]. Reactivation of trigeminal nerve HSV latency is thus the likely pathogenesis of delayed lesions, which occur 2–7 days after UVR exposure (figure 1). However, the pathogenesis of the immediate lesions, particularly those that appear \(<24\) h after the stimulus, is more problematic. Possible explanations for immediate lesions include an anatomic location of viral latency near or within the labial epidermis; “hyperinfection” of the trigeminal ganglia such that reactivation results in a larger viral inoculum or more rapid spread of virus to the lip; or as-yet-undetectable immunologic abnormalities in those with immediate lesions such that viral reactivation and spread occurs more readily. Another possibility is that the immediate lesions represent viral reactivation from ganglionic sites other than the trigeminal ganglion, such as the pterygopalatine or submandibular ganglia.

The ACV trials in this report strengthen four emerging concepts concerning the management of herpes labialis with antiviral compounds [5, 6, 9]: Prophylactic peroral ACV is only partly effective against UVR-induced herpes labialis because the immediate lesion subgroup cannot be prevented, treatment of herpes labialis with antiviral compounds will not “abort” lesions, treatment of herpes labialis with antiviral compounds can reduce lesion severity, and peroral ACV is superior to topical ACV cream and ointment for the prophylaxis and treatment of herpes labialis.

In our first trial (table 1), ACV or placebo capsules were administered prophylactically to experimental UVR-exposed volunteers in an attempt to confirm our earlier finding that ACV prevented herpes labialis in skiers [9]. The overall frequency of experimental lesions was reduced from 39% in the placebo group to 18% among ACV recipients, comparable to the reduction in lesion frequency by peroral ACV among the sunlight-exposed skiers (26% to 7%). In addition, we saw
the same pattern of lesion susceptibility in the experimental and the field trials: Lesions developing soon after the inducing stimulus were not prevented by ACV prophylaxis, whereas delayed-onset lesions were prevented. Because in half of the experimental subjects prophylactic therapy was started 7 days before UVR exposure and immediate lesions still developed among ACV recipients, it is unlikely that the immediate lesions were HSV reactivations coincidentally “in progress” in some patients at the time of UVR exposure.

To study the efficacy of peroral ACV when taken as an early, patient-initiated treatment of a new herpes episode, at the time of the first local premonitory sign or symptom, ACV or placebo capsules were administered 48 h after UVR, just before the usual appearance of the delayed lesion subpopulation (table 1, early treatment). Antiviral treatment did not reduce the total number of delayed lesions or increase the frequency of papular (aborted) lesions. In contrast, the severity of treated lesions was markedly reduced among ACV recipients, manifested by 40%-60% decreases in mean lesion size and healing time in comparison with the placebo group (P = .01-05).

Our findings with peroral ACV for the treatment of UVR-induced herpes labialis are consistent with the data from two recent field trials of peroral ACV. In these studies, early, patient-initiated treatment of new episodes of herpes labialis reduced lesion severity but did not affect the number of lesions that developed [5, 6]. The “jump” on starting therapy made possible under experimental circumstances likely was responsible for the greater reduction in lesion severity in the present study than was seen in the field trials, but it is noteworthy for future studies that prevention of UVR-induced lesions was not accomplished even though treatment was initiated before the onset of any symptoms. It is possible that higher peak plasma levels of ACV than can be obtained with our peroral regimen (200 mg [five times/day], 0.5 μg/ml [22]) would more successfully inhibit lesion formation.

Our negative results with 5% ACV cream increase the evidence that topical cream and ointment formulations of ACV have only marginal clinical activity [1-4, 7, 8, 23-25]. Because of the relatively few patients with lesions in the present study, a small reduction in lesion severity may have occurred that we were unable to detect. Drug delivery through the skin may have been inadequate, either because the flux of ACV through the skin from the new cream vehicle was too little or, depending on the degree of “feel” of the formulation, patients may have been stimulated to lick their lips and remove the medication. In one study, cream- and grease-based sunscreens were objectionable to volunteers on this basis and provided only 27% protection against sunburn after 1 h [26]. Our studies indicate that ACV capsules can prevent and treat herpes labialis and should be considered the preferred route of administration until the problems associated with topical administration have been resolved.

References

