Iontophoresis of Vidarabine Monophosphate for Herpes Orolabialis

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The cutaneous application of antiviral agents was studied by iontophoresis, a process that increases penetration of most drugs 20- to 60-fold. Twenty-seven subjects with vesicular orolabial herpes were treated one time in a double-blind, placebo-controlled clinical study: nine received vidarabine monophosphate (ara-AMP), nine received acyclovir (ACV), and nine received NaCl. Ara-AMP-treated lesions yielded lower titers of virus after 24 hr compared with lesions treated with NaCl or ACV \( (P < .05) \). Ara-AMP significantly decreased the duration of shedding of virus \( (P < .05) \) and time to dry crust \( (P < .05) \) compared with the other two agents. There was a trend toward decreased healing time after ara-AMP treatment.

The treatment of infections due to herpes simplex virus (HSV) has been the subject of many research studies and anecdotal reports. Most attempts to prove the efficacy of topical treatment of recurrent cutaneous infections due to HSV have failed [1, 2]. Because many nucleoside analogues are effective in vitro and are generally successful in topical therapy for herpes keratitis (corneal infections of the eye), the most probable explanation for treatment failure in cutaneous tissues is lack of penetration.

On the basis of animal studies [3-6] and rapid resolution of recurrent orolabialis lesions in humans after iontophoresis with idoxuridine (Stoxil™; Smith Kline & French, Philadelphia) in open trials [7], we proposed that iontophoresis could overcome the cutaneous barrier by propelling ionic antiviral agents into surface tissue and result in efficacy of the drugs against cutaneous infections with HSV types 1 and 2. We report here a controlled, double-blind clinical trial of iontophoresis with vidarabine monophosphate (ara-AMP), acyclovir (ACV), and NaCl (placebo).

Subjects and Methods

Subjects with recurrent herpes orolabialis were recruited. The presence of HSV vesicular lesions on the mucocutaneous junction surrounding the mouth made the patient eligible for the study. Pregnant women, children, and individuals in poor health were excluded. The subject population consisted of 18 women and nine men (mean ages, 27-32 years; mean lesion durations, 24-29 hr). Thirteen subjects had frequent lesions, and 14 had infrequent lesions (fewer than three recurrences in the previous nine months). Although there were slight variations in these demographic characteristics between groups, there were no statistically significant differences between groups in any of these parameters.

Drugs. Ara-AMP sodium salt was supplied by Dr. Edwin L. Marcus (Warner-Lambert/Parke Davis, Ann Arbor, Mich) and ACV disodium salt by Dr. Dannie King (Burroughs-Wellcome, Research Triangle Park, NC). NaCl was purchased from Sigma Chemical Co. (St. Louis). The powders were desiccated, weighed, and placed in vials so that the later addition of 2 ml of distilled water resulted in an iso-
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Iontophoresis. The masked drug vial was opened, and 2 ml of sterile distilled, deionized water was added. After solubilization the contents of the vial were placed in a 1-oz plastic cup. The drug delivery system used (ElectroApplicator System™ model C-2; Dentelect Corp., Augusta, Ga) and the method of application have been described previously [8]. A plastic tube 1 cm in diameter filled with cotton was placed in the drug solution so that it soaked into the cotton. This device (the treatment electrode) was placed over the lesion and overlapped all borders by at least 2 mm. The metal end of a flexible vinyl probe was engaged against the cotton, and the other end of the probe was connected to the negative terminal (cathode) of the iontophoresis unit. The return electrode (anode) included an adhesive bandage-like device consisting of a paper disk saturated with sodium nitrate that was attached to the volar surface of the midforearm.

The iontophoresis module used as the power source in the ElectroApplicator System was the Phoresor® (model PM-600; Motion Control, Salt Lake City). This unit is capable of operating from 0 to 60 V DC with a 9-V battery source with a step-up transformer. The current was allowed to run at 0.5–0.7 mA for 6–8 min and resulted in an electrical charge flow of 4.0 mA-min or 0.24 A-sec. Larger lesions required an extra piece of cotton soaked in the drug before placement of the treatment electrode and additional current time in proportion to surface area covered. After treatment the subject was asked to keep the lesion dry as much as possible and avoid application of ointments, coatings, or medications.

Evaluation. The lesion was rephotographed 10 min after treatment, and any changes in posttreatment signs and symptoms were noted. The subjects returned after 24 and 48 hr; at least five times within the first seven days; then once on day 9, 10, or 11; and once on day 19, 20, or 21, for a total of eight visits. At each visit the photography was repeated, the lesion was evaluated for symptomatology, and collection of virus was repeated on as many days as possible. If the lesion bled during teasing, it was considered to be in the scab stage, and swabbing for virus at later appointments was not attempted.

Detection of virus. After the period of shaking

Virus (0.15 M). The final weight/volume concentrations were 5.22% for ara-AMP, 3.75% for ACV, and 0.9% for NaCl; these concentrations provide the same number of ions per milliliter of solution. Each vial was wrapped in aluminum foil so that the solubilization reaction could not be observed by the operator. Vials were coded with a 6-digit random number, placed in a desiccator for 24 hr, capped, and tightly sealed with a triple covering of Parafilm® (American Can Co., Greenwich, Conn). Vials were stored at room temperature (~23 C) in sets of nine, with three vials of each drug per set.

Drug assignment. Subjects selected a randomly coded vial from a set of nine; when the set was used, a new set of nine was started. A balanced sample of 27 subjects was obtained by enrollment of a total of 41 patients. This sample provided a study population of 27 subjects (nine treated by ara-AMP, nine by ACV, and nine by NaCl), all of whom had vesicular HSV that was verified by positive culture of virus. The remaining 14 subjects who drew vials were excluded from the study for the following reasons: (1) Three subjects showed no virus in the pretreatment sample. (2) One subject was inadvertently admitted with a nonvesicular (papular) lesion. (3) Three subjects had to be replaced because their drug (ACV after several months' storage) formed a precipitate on the lip. (4) Three subjects (two receiving ACV and one receiving NaCl) chose vials that were included to preserve the blinding. (5) One subject did not comply with study directions. (6) Three subjects failed to complete the study.

Subjects. Histories, including general health, specifics of past HSV lesions, and present symptomatology, were obtained. Subjects with two or fewer lesions in the previous nine months were assessed as having infrequent lesions, whereas three or more lesions in nine months was considered frequent. Lesions were photographed with a millimeter scale in place, categorized, and rated for appearance.

The lesion was gently washed with sterile 0.9% NaCl, dried with a sterile swab, and opened aseptically with a scalpel. Vesicular fluid was adsorbed by pressing and partially rotating a Dacron® (Dupont, Wilmington, Del) swab three times on the lesion so that the skin blanched. The swab was transferred aseptically to a tube containing Eagle's MEM and placed in a shaker for 1½ hr at 37 C before further processing (see detection of virus below).
in Eagle's MEM, the swab was transferred to a tube containing a primary rabbit kidney (PRK) monolayer. The balance of the transport medium was frozen and stored at -80°C for subsequent titration of virus. The contents of the PRK monolayer tube, containing Eagle's MEM with 2% fetal bovine serum and an antibacterial mixture, were incubated at 35°C in an atmosphere of 5% CO₂ and 95% air. After 24 hr the swab was removed, and 1.0 ml of Eagle's MEM plus fetal bovine serum was added for pH adjustment and nutrition. The monolayer was observed once a day for 10 days for detection of CPE. If the PRK monolayer culture was positive, plaque assays were done with CV-1 cells. Serial log dilutions of the original frozen sample were made on six-well plates. Samples positive for virus were checked in triplicate at each 10-fold dilution until <50 pfu per well were obtained. This number of pfu was easy to count and was always achieved in six dilutions (10⁻⁶). If there was no CPE in the PRK monolayer after 10 days, the swab was considered negative for virus. However, all such samples negative for virus were rechecked with use of 0.5 ml of the frozen stored samples. If subsequent CPE was observed after plating on PRK monolayers in six-well plates, the samples were assessed as positive and scored as having 2 pfu/ml. Cultures from the original swab that were positive (containing 0.2 ml) from the PRK monolayer tubes but negative on CV-1 cells were rated as having 5 pfu. All other pfu readings were taken from the plaque assays.

**Results**

The mean lesion areas at the initial examination were not statistically different among treatment groups (data not shown). There were no statistical differences in pain scores before or after treatment among groups (data not shown). No adverse effects of any of the treatments were noted.

Titers of virus from the plaque assay on CV-1 cells are shown in table 1, where statistically significant comparisons are also noted. The data were first analyzed by an analysis of variance, which indicated a significant reduction in number of pfu with time (P < .001). Individual comparisons (within group t tests) were performed for reductions with time. Ara-AMP-treated lesions had significantly fewer pfu at 24 hr (3.80 log reduction in pfu; P < .001) and 48 hr (4.38 log reduction; P < .001) hr compared with pretreatment titers, as well as a significant reduction in number of pfu between 24 and 48 hr (0.59 log reduction; P < .05). In ACV-treated lesions the decrease in titer of virus was significant between pretreatment and 48 hr (2.42 log reduction; P < .05) but not at other times. Titers of virus from NaCl-treated lesions was also reduced more slowly than for ara-AMP-treated lesions; there were significant differences between pretreatment and 48 hr (3.91 log reduction; P < .001) and between 24 and 48 hr (1.91 log reduction; P < .05) but not between pretreatment and 24 hr. Analysis of the number of pfu after different treatments disclosed that (1) all groups started at approximately the same titer, (2) the results of ACV and NaCl treatment were not significantly different, and (3) the log number of pfu was significantly reduced for ara-AMP-treated lesions compared with ACV- or NaCl-treated lesions at 24 hr (P < .05 by Student's t test).

The titer of virus at 48 hr was also reduced for ara-AMP treatment compared with ACV treatment (P < .05).

The mean change in lesion symptoms for the three treatments is given in table 2. Mean number of days to dry crust from onset time was significantly decreased for ara-AMP treatment (2.4 days; P < .005) compared with ACV (5.2 days) and NaCl (4.8 days) treatment. ACV- and NaCl-treated lesions did not differ. The results of analysis for virus on PRK monolayers are also given in table 2. Differences in duration of shedding of virus (in hr) from onset time to virus-free lesion (no CPE on PRK monolayers) among the groups were statistically significant (P < .05 by Kruskal-Wallis test). Further statistical analysis (Mann-Whitney test) indicated ara-AMP-treated lesions had significantly shorter intervals of shedding of virus compared with ACV- or NaCl-treated lesions (P < .05). Ara-AMP-treated lesions healed in ~80% of the time required for lesions treated with

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>24 hr</th>
<th>48 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara-AMP</td>
<td>4.6 ± 0.6</td>
<td>0.8 ± 0.2*</td>
<td>0.2 ± 0.1†</td>
</tr>
<tr>
<td>ACV</td>
<td>4.6 ± 0.7</td>
<td>3.0 ± 0.8</td>
<td>2.2 ± 0.9‡</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.0 ± 0.3</td>
<td>3.0 ± 0.9</td>
<td>1.1 ± 0.6§</td>
</tr>
</tbody>
</table>

NOTE. Data are mean ± SE log no. of pfu. Statistical significance was calculated by paired t test.

* P < .001 for reduction from baseline, P < .05 compared with ACV, and P < .05 compared with NaCl.
† P < .001 for reduction from baseline, P < .05 for reduction from 24 hr, and P < .05 compared with ACV.
‡ P < .05 for reduction from baseline.
§ P < .001 for reduction from baseline, and P < .05 for reduction from 24 hr.
ACV or NaCl, but this difference was not statistically significant.

Discussion

This study offers evidence that one iontophoretic treatment with ara-AMP is effective therapy for orolabial HSV. The most important parameters of antiviral effectiveness — reduction in titer and elimination of virus and reduced time to dry crust — were significantly improved with ara-AMP compared with ACV and placebo. The study involved nine subjects per treatment group; nevertheless, the demonstration of statistical significance in many important quantitative parameters between such small groups of subjects indicates a highly effective therapy.

Both ara-AMP [9] and ACV [10, 11] have been shown to be ineffective in topical treatment of recurrent cutaneous infections with HSV. We believe the delivery method used in our study, i.e., iontophoresis, accounts for the observed effectiveness. The direct current of iontophoresis increases cutaneous penetration of antiviral agents 20- to 60-fold [12]. Iontophoretic treatment of HSV lesions in animal studies [3–6] indicated that electrical delivery of highly ionized antiviral drugs was worthy of clinical trials in humans.

Vidarabine and ACV are both approved for human use. Vidarabine was approved for iv use in herpes encephalitis and varicella zoster and for topical application in herpes keratitis [13]. Ara-AMP, the monophosphate derivative of vidarabine, was tested in humans [14] because its high solubility favors iv delivery. We used ara-AMP because its highly ionic nature is ideal for delivery by iontophoresis [4, 12]. Spruance et al. [9] tested 10% ara-AMP gel in recurrent cutaneous infection with HSV by routine topical application and found no difference from placebo. Our earlier animal studies assessed iontophoretic delivery of ara-AMP in HSV-infected mouse skin [4], guinea pig skin [6], and rabbit eyes [5]. Iontophoretic delivery of ara-AMP was superior to topical or iontophoresic delivery of several other nucleoside analogues, including ACV and idoxuridine.

Acyclovir was introduced by Elion et al. [15] and was being examined for efficacy against cutaneous HSV when our animal studies were in progress. Because ACV is supplied as a disodium salt, we used it for iontophoresis. Our earlier animal tests [5] indicated that ACV was active when applied iontophoretically, but no difference from placebo in humans was found with application by iontophoresis in the current study. This lack of effect may relate to an excessively high solution pH of 10.6, which favors introduction of OH− rather than ACV. Another possibility is a β statistical error; the possibility of β error increases with lower sample size. However, the results obtained favor the use of ara-AMP for iontophoresis and for further trials. Recent studies have examined the routine topical application of ACV preparations. Early topical application of ACV cream to cutaneous lesions is, at best, marginally effective [16–18], whereas other studies could not demonstrate any statistical difference from placebo, irrespective of when therapy was started [10, 11].

Our present studies did not assess repetitive iontophoretic therapy, and thus they are not directly comparable with repetitive oral drug therapy. ACV in any dosage form is inactive or at best only minimally effective [16–18] as therapy for active recurrent, cutaneous lesions. Furthermore, both extensive topical application and long-term oral acyclovir may produce viral resistance [19, 20]. Although prophylactic, oral ACV is useful in decreasing the occurrence of recurrent genital herpes [20], such a strategy has not been studied and is probably not indicated for decreasing recurrent orolabial herpes. Also, the long-term adverse effects of continuous therapy with ACV are unknown, and its continuous use is expensive. We believe there is a need for additional forms of antiviral therapy, especially for active HSV cutaneous lesions, for which no therapy is currently satisfactory. A single treatment with iontophoretic delivery of ara-AMP offers an additional attractive choice in therapy for cutaneous HSV. Ara-AMP therapy was particularly effective in the rapid elimination of virus and greatly reduced the potential risks associated with systemic treatment.


