Comparison of New Topical Treatments for Herpes Labialis

Efficacy of Penciclovir Cream, Acyclovir Cream, and n-Docosanol Cream Against Experimental Cutaneous Herpes Simplex Virus Type 1 Infection

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Background: There are 3 new topical treatments for herpes labialis that have either been approved by the US Food and Drug Administration (penciclovir cream [Denavir] and n-docosanol cream [Abreva]) or recently undergone extensive clinical evaluation (acyclovir cream). The relative efficacy of these products is unknown.

Objective: To compare the efficacy of penciclovir cream, acyclovir cream, n-docosanol cream, and acyclovir ointment in an experimental animal model of cutaneous herpes simplex virus type 1 (HSV-1) disease.

Design: The backs of guinea pigs were infected with HSV-1 using a vaccination instrument. Active treatments and corresponding vehicle controls were applied for 3 to 5 days beginning 24 hours after inoculation.

Main Outcome Measures: After completion of treatment, the animals were killed and the severity of the infection assessed from the number of lesions, the total lesion area, and the lesion virus titer.

Results: Penciclovir cream effected modest reductions in lesion number (19%), area (38%), and virus titer (88%) compared with its vehicle control, and each of these differences was significantly greater ($P<.05$) than the reductions effected by acyclovir ointment (0%, 21%, and 75%, respectively). The acyclovir cream effect (reductions of 4%, 28%, and 77%, respectively) was less than that of penciclovir cream, and this difference was confirmed by 2 additional head-to-head experiments. Two experiments with n-docosanol cream failed to show statistically significant differences by any parameter between n-docosanol cream and vehicle control–treated sites or between n-docosanol and untreated infection sites.

Conclusions: In this model, the efficacy of penciclovir cream was greater than acyclovir cream, acyclovir cream was greater than or equal to acyclovir ointment, and acyclovir ointment was greater than n-docosanol cream. Since our model was designed to evaluate compounds that function primarily through antiviral activity, the negative findings with n-docosanol in these studies do not exclude that it might work clinically through other mechanisms.

Arch Dermatol. 2001;137:1153-1158

Following a period of uncertainty regarding efficacy, antiviral treatments for herpes labialis have been approved by regulatory agencies in the United States and other countries. In the United States, acyclovir ointment (Zovirax Ointment) is approved for use in immunocompromised patients and penciclovir cream (Denavir) for use in otherwise healthy adults. In other countries, 1 or more ethical drug treatments, including penciclovir cream, acyclovir cream, and idoxuridine in dimethyl sulfoxide, are generally available. There is experimental evidence that high-dose peroral nucleoside analogue therapy may be highly effective, possibly because of delivery of high concentrations of drug to the site of the infection. It is unlikely that the relative clinical efficacy of these various treatments will ever be determined. The dorsal cutaneous guinea pig model is a well-standardized animal model of cutaneous herpes disease and permits an experimental approach to this question. Drug efficacy in the model correlates

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Because of the large number of patients and the expense required to conduct a therapeutic trial in herpes labialis, it is unlikely that the relative clinical efficacy of these various treatments will ever be determined. The dorsal cutaneous guinea pig model is a well-standardized animal model of cutaneous herpes disease and permits an experimental approach to this question. Drug efficacy in the model correlates
MATERIALS AND METHODS

ANTIVIRAL AGENTS

Both 10% and 12% n-docosanol cream and 10% and 12% stearic acid-supplemented vehicle controls were provided by Lidak Pharmaceuticals (La Jolla, Calif). The 10% n-docosanol cream is now marketed over the counter as Abreva by GlaxoSmithKline (Research Triangle Park, NC). The 5% acyclovir cream (Zovirax Cream [Europe]), 5% penciclovir cream (Denavir [United States], Vectavir [Europe]), and their corresponding vehicles were provided by GlaxoWellcome (Research Triangle Park, NC). The 1% penciclovir cream (Denavir [King of Prussia, Pa]) and its vehicle were provided by SmithKline Beecham (Philadelphia, Pa).

EXPERIMENTAL VIRUS STRAIN

The virus was the laboratory strain HSV-1 E115, originally obtained from Andre Nahmias, MD, MPH (Emory, Ga), and used in our model since 1980.11 Virus stock for inoculation of guinea pigs contained 1 × 10⁶ plaque-forming unit (pfu)/mL and was prepared in mink lung cells (MV-1-Lu, American Type Culture Collection).

EXPERIMENTAL ANIMALS

Guinea pigs were inoculated with HSV-1 E115 (day 0) in 4 different areas on the depilated dorsum, right and left midback, and right and left rump by multiple shallow punctures with a vaccination instrument as originally performed by Hubler et al.12 Between 40 and 60 discrete lesions developed at each of the 4 infection sites. Test treatments were begun 24 hours after inoculation (day 1) and given 1 to 4 times per day at 8 AM, noon, 4 PM, and 8 PM, depending on dermal irritation, for a total of 3 days. The day after completion of treatment (day 4), the dorsum of each animal was again depilated, and the severity of infection at each site was assessed from the number of lesions, the total lesion area, and titer of virus in the excised infection site. The procedures have been described in detail elsewhere.12

STATISTICAL PROCEDURES

Paired data (drug and drug vehicle) were evaluated by the Wilcoxon signed rank test. To compare 2 drugs with one another, efficacies were expressed as the percent difference in a lesion severity measure (lesion number, area, or virus titer) between the active formulation and its control and tested by a Mann-Whitney rank sum procedure. All probability determinations were 2-tailed, and P≤.05 was considered significant.

RESULTS

PENCICLOVIR CREAM, ACYCLOVIR CREAM, AND ACYCLOVIR OINTMENT VS VEHICLE CONTROLS

Penciclovir and acyclovir creams were examined for efficacy in relationship to their corresponding vehicles, and their vehicles were each applied at 3 different sites once per day, 2 times per day, and 4 times per day for 3 days and evaluated for irritation on the morning of the fourth day using an irritation score of 0 to 4. A score of 4 indicated severe erythema with punctate bleeding. A mean score of 2.5 or greater indicated that the compound and/or its vehicle at that dosing frequency caused sufficient irritation to compromise assessment of the viral infection and potentially affect the outcome of the experiment.

ANIMAL INOCULATION AND TREATMENT

Guinea pigs were inoculated with HSV-1 E115 (day 0) in 4 different areas on the depilated dorsum, right and left midback, and right and left rump by multiple shallow punctures with a vaccination instrument as originally performed by Hubler et al.12 Between 40 and 60 discrete lesions developed at each of the 4 infection sites. Test treatments were begun 24 hours after inoculation (day 1) and given 1 to 4 times per day at 8 AM, noon, 4 PM, and 8 PM, depending on dermal irritation, for a total of 3 days. The day after completion of treatment (day 4), the dorsum of each animal was again depilated, and the severity of infection at each site was assessed from the number of lesions, the total lesion area, and titer of virus in the excised infection site. The procedures have been described in detail elsewhere.12

STATISTICAL PROCEDURES

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RESULTS

PENCICLOVIR CREAM, ACYCLOVIR CREAM, AND ACYCLOVIR OINTMENT VS VEHICLE CONTROLS

Penciclovir and acyclovir creams were examined for efficacy in relationship to their corresponding vehicles,
between acyclovir and penciclovir cream reached statistical significance for virus titer (P < .05).

**PENCICLOVIR CREAM VS ACYCLOVIR CREAM**

To further examine the potential differences between penciclovir and acyclovir cream, the 2 treatments were compared directly with one another in 2 additional experiments. Treatments were only applied once a day because acyclovir and penciclovir cream were irritating to guinea pig skin. The results are shown in Figure 1. In both experiments, penciclovir cream–treated sites had fewer mean numbers of lesions than at acyclovir cream–treated sites (46–49 vs 52–56, respectively) and a smaller mean lesion area (197-219 vs 258-280 mm², respectively). Three of these 4 differences were statistically significant (P < .05). There was no difference between the 2 treatments in the mean lesion virus titer.

**Table 1. Penciclovir Cream Treatment vs Vehicle Control Treatment in the Guinea Pig Model**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Penciclovir</th>
<th>Difference Between Means, % (P Value)</th>
<th>Vehicle Control</th>
<th>Acyclovir</th>
<th>Difference Between Means, % (P Value)</th>
<th>Vehicle Control</th>
<th>Acyclovir Ointment</th>
<th>Difference Between Means, % (P Value)</th>
<th>Vehicle Control</th>
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<tr>
<td>No. of lesions</td>
<td>Mean</td>
<td>39 (0.04)‡</td>
<td>48</td>
<td>50</td>
<td>4 (0.06)‡</td>
<td>52</td>
<td>49</td>
<td>-6 (0.18)</td>
<td>46</td>
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<tr>
<td></td>
<td>SD</td>
<td>11</td>
<td>7</td>
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<td>4</td>
<td>11</td>
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<td>11</td>
<td>12</td>
<td>12</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>41</td>
<td>49</td>
<td>52</td>
<td>52</td>
<td>49</td>
<td>48</td>
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<tr>
<td>Total lesion area, mm²</td>
<td>Mean</td>
<td>150 (0.02)‡</td>
<td>21</td>
<td>205</td>
<td>28 (0.008)§</td>
<td>283</td>
<td>182</td>
<td>21 (0.004)§</td>
<td>230</td>
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<td></td>
<td>SD</td>
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<td>44</td>
<td>59</td>
<td>67</td>
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</tr>
<tr>
<td></td>
<td>Median</td>
<td>151</td>
<td>231</td>
<td>185</td>
<td>295</td>
<td>170</td>
<td>235</td>
<td></td>
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<tr>
<td>Virus titer, log₁₀ pfu/mL</td>
<td>Mean</td>
<td>4.1</td>
<td>5.0</td>
<td>5.7</td>
<td>5.2</td>
<td>4.2</td>
<td>75 (0.002)‡</td>
<td>4.8</td>
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<tr>
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<td>SD</td>
<td>0.5</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
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<tr>
<td></td>
<td>Median</td>
<td>4.1</td>
<td>5.0</td>
<td>5.4</td>
<td>5.1</td>
<td>4.3</td>
<td>4.9</td>
<td></td>
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</tr>
</tbody>
</table>

*Treatments were administered once per day for 3 days. pfu indicates plaque-forming unit.
‡Significantly more effective than acyclovir ointment (P < .05).
§Significantly more effective than acyclovir cream (P < .05).

**Figure 1.** Penciclovir cream and acyclovir cream were compared directly with one another in 2 separate experiments. The 2 treatments were applied opposite each other at 12 contralateral infection sites in experiment 1 and 16 sites in experiment 2. There were no vehicle controls. The treatments were only applied once a day because acyclovir and penciclovir cream were irritating to guinea pig skin. pfu indicates plaque-forming unit. The asterisk designates when the difference between penciclovir cream and acyclovir cream was statistically significant.

**n-Docosanol Cream vs Acyclovir Ointment**

Two separate experiments were done to evaluate the potential efficacy of n-docosanol cream. Because the formulation lost its consistency when n-docosanol was removed, the sponsor provided a vehicle control in which stearic acid was added, which gave the preparation a similar turgor and appearance to the active formulation. The vehicle with stearic acid proved to be highly irritating and could only be applied once per day, whereas n-docosanol cream was tolerated at a maximum dosing frequency of 3 times per day. After consultation with the sponsor, the first experiment was performed in which 10% n-docosanol cream was applied 3 times per day for 3 days and compared with untreated infection sites. Acyclovir ointment and ointment control were applied 4 times per day for 3 days, our standard comparator regimen in this model. The results are shown in Table 2. Acyclovir ointment effected statistically significant reductions in all le-
sion parameters compared with the ointment vehicle control, whereas n-docosanol had no effect. The amount of reduction in the mean lesion area by acyclovir ointment was 22%.

To further explore the potential activity of n-docosanol, a second experiment was performed in which the concentration of n-docosanol was increased to 12%, the duration of treatment was increased from 3 to 5 days, and efficacy parameters were assessed on 3 days (days 4, 5, and 6) instead of just on day 4. The 12% stearic acid–supplemented cream was used as the vehicle control. Acyclovir ointment and ointment control were again included as comparators. All treatments were applied 3 times per day for 5 days. The results are shown in Figure 2. Acyclovir ointment was effective on all 3 days, whereas n-docosanol showed no benefit. The amount of reduction in the mean lesion area by acyclovir ointment on days 4, 5, and 6 was 23%, 31%, and 28%, respectively.

### Comment

The relative efficacy of the treatments tested in this study was penciclovir cream greater than acyclovir cream greater than or equal to acyclovir ointment greater than n-docosanol cream. Total lesion area, the major clinical parameter in the guinea pig model, was reduced 38%, 28%, 21%, and 0%, respectively, by these treatments. Prior studies with 15% idoxuridine in dimethyl sulfoxide in this model have shown a reduction in lesion area of 80%. Each of these products has also been evaluated in clinical trials as therapy for herpes labialis with lesion healing time as the primary efficacy variable. The efficacy values for these 5 treatments in the guinea pig model and in clinical trials against herpes labialis in normal hosts are compared in Table 3.

As shown in Table 3, the rank order of results with different compounds in our model generally parallels the rank order of results in clinical trials. Infection in the model is roughly twice as sensitive to the antiviral treatments as is herpes labialis and so exaggerates the degree of efficacy compared with the outcome in the human condition. This is, in part, because dorsal cutaneous HSV guinea pig disease is a primary infection, whereas herpes labialis is a recrudescence. In the latter instance, the secondary immune response truncates the course of the illness and abbreviates the window of opportunity for chemotherapy. Awan et al21 have convincingly demonstrated the negative impact of the immune response on drug efficacy by studies of topical anti-HSV therapy in mice with and without the adoptive transfer of HSV-sensitized mononuclear cells.

It is possible that the dosing regimen of acyclovir cream was not optimal in our animal experiments. Specifically, more frequent dosing of acyclovir cream might have been associated with greater efficacy. The potential impact of dosing frequency on comparative studies of penciclovir and acyclovir in animal models has been previously reported22 and may be related to differences in the stability of the intracellular triphosphate metabolites of the 2 drugs. In the present experiments, skin irritation limited treatment applications to once a day.

Evidence from the experimental model and in vitro studies of drug penetration through animal and human skin samples21,23 showed that better drug delivery significantly improved efficacy and that we were in the early portion of a dose-response curve with our current therapies for cutaneous HSV infection. To some extent, this is apparent in the clinical trials of topical formulations (Table 3). To further substantiate this principle in herpes labialis, we compared 3 different doses of peroral famciclovir in the treatment of UV radiation–induced recurrences. There was a significant dose-response effect, indicating that higher peroral doses of antiviral agents or topical formulations providing improved intraepidermal drug penetration are strategies that should be explored in the treatment of herpes labialis.7

### Table 2. n-Docosanol Cream vs Acyclovir Ointment Treatment in the Guinea Pig Model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Docosanol Cream†</th>
<th>Difference Between Means, % (P)</th>
<th>Untreated</th>
<th>Acyclovir Ointment‡</th>
<th>Difference Between Means, % (P)</th>
<th>Ointment Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of lesions</td>
<td>Mean</td>
<td>56</td>
<td>-2 (.68)</td>
<td>55</td>
<td>54</td>
<td>5 (.008)</td>
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<tr>
<td>SD</td>
<td>3</td>
<td></td>
<td>3</td>
<td>4</td>
<td></td>
<td>3</td>
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<tr>
<td>n</td>
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<td>12</td>
<td>12</td>
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</tr>
<tr>
<td>Median</td>
<td>56.5</td>
<td></td>
<td>55</td>
<td>55.5</td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>Total lesion area, mm²</td>
<td>Mean</td>
<td>232</td>
<td>8 (.07)</td>
<td>252</td>
<td>188</td>
<td>22 (.004)</td>
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<tr>
<td>SD</td>
<td>35</td>
<td></td>
<td>31</td>
<td>40</td>
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<td>27</td>
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<td>n</td>
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<td></td>
<td>12</td>
<td>12</td>
<td></td>
<td>12</td>
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<tr>
<td>Median</td>
<td>245</td>
<td></td>
<td>264</td>
<td>177</td>
<td></td>
<td>245</td>
</tr>
<tr>
<td>Virus titer, log pfu*/mL</td>
<td>Mean</td>
<td>4.88</td>
<td>0 (.70)</td>
<td>4.87</td>
<td>4.19</td>
<td>72 (.002)</td>
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<tr>
<td>SD</td>
<td>0.32</td>
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<td>0.28</td>
<td>0.27</td>
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<td>0.31</td>
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<tr>
<td>Median</td>
<td>4.98</td>
<td></td>
<td>4.91</td>
<td>4.19</td>
<td></td>
<td>4.85</td>
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</table>

*pfu indicates plaque-forming unit.
†Administered 3 times per day for 3 days.
‡Administered 4 times per day for 3 days.
There has been considerable controversy recently concerning the potential effect of the vehicle comparator arm on the apparent efficacy of topical antitherpetic compounds in clinical trials.18,24 Treatment advocates have claimed that the vehicles have efficacy, therefore reducing the overall benefit of the formulation compared with an untreated control arm. Potential mechanisms of vehicle efficacy include antiviral activity, acceleration of wound healing by occlusion, and the eschar-dissolving potential of vehicle solvents such as propylene glycol. It is also possible that drug vehicles could have an adverse effect on lesion severity, for example, by skin irritation, such that the efficacy of a treatment would be exaggerated by drug-vehicle comparisons. These issues are best examined experimentally, because the placebo effect in human studies makes it difficult to interpret a trial in which one group of patients has not received treatment.

The issue of vehicle effects is of importance in the case of n-docosanol cream. The n-docosanol cream formulation loses its consistency when n-docosanol is omitted. To conduct clinical trials, it was necessary to have a vehicle that matched the active formulation in appearance, and this was accomplished by adding stearic acid. A small pilot trial (n=63) that used a stearic acid–containing vehicle control showed efficacy,25 but a subsequent much larger study (n=846) found no benefit.17 Because of a concern that the vehicle may have had a beneficial effect on herpes labialis and masked an effect by n-docosanol, a polyethylene glycol control formulation was prepared. The clinical trials were repeated using polyethylene glycol as the control, and a 15% reduction in lesion healing time in the n-docosanol arm recently was reported.18 The differing trial results and the complicated issue of vehicle effects as a potential explanation for the differences make it hard to evaluate this product.

In the present studies, n-docosanol cream was examined in several ways. When compared against untreated lesion sites, no clinical or antiviral activity was seen (Table 2). When compared against a stearic acid–containing vehicle on multiple days, again no clinical or antiviral activity was seen (Table 2). When compared against a stearic acid–containing vehicle on multiple days, again no clinical or antiviral activity was seen. In the latter experiment, acyclovir ointment and ointment control (polyethylene glycol–containing placebo) were run as comparators. There were no significant differences in any measure between the stearic acid–containing vehicle control and polyethylene glycol control formulation. One group of patients has not received treatment.

### Table 3. Topical Antiviral Drug Efficacy in Herpes Labialis and in Experimental Dorsal Cutaneous Herpes Simplex Virus Infection in the Guinea Pig

<table>
<thead>
<tr>
<th>Topical Antiviral Drug Formulation</th>
<th>Efficacy in the Guinea Pig Model, %†</th>
<th>Efficacy in Herpes Labialis, %‡</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Docosanol cream</td>
<td>4-8</td>
<td>0/15</td>
<td>17, 18</td>
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<tr>
<td>Acyclovir ointment</td>
<td>21-22</td>
<td>Nonsignificant</td>
<td>1, 15, 16</td>
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<tr>
<td>Acyclovir cream</td>
<td>28</td>
<td>10-125</td>
<td>19</td>
</tr>
<tr>
<td>Penciclovir cream</td>
<td>38</td>
<td>13-175</td>
<td>20</td>
</tr>
<tr>
<td>Idoxuridine in dimethyl sulfoxide</td>
<td>80</td>
<td>38</td>
<td>6, 14</td>
</tr>
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</table>

*Reduction in total lesion area on day 4.
†Reduction in healing time of classic lesions treated early.
‡Trial with stearic acid–containing placebo/trial with polyethylene glycol–containing placebo.
§Includes some cases treated late.
||There was no difference between patients treated early or late.

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Figure 2. Comparison of 12% n-docosanol and acyclovir ointment with vehicle controls at 3 different time points. The vehicle control for n-docosanol was a 12% stearic acid–supplemented cream. There were 12 infection sites per treatment group. Day 0 was the day of infection. The treatments were applied 3 times per day for 3 to 5 days beginning on day 1. Efficacy measurements were done on the morning of days 4, 5, and 6. Assessments of lesion virus titers were only performed on day 6. pfu indicates plaque-forming unit. The asterisk designates when the difference between the test drug and its vehicle control was statistically significant.
REFERENCES


