Therapeutic Effect of Trisodium Phosphonoformate on
Cutaneous Herpesvirus Infection in Guinea Pigs

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When applied topically, trisodium phosphonoformate (PFA) displayed activity against established cutaneous herpesvirus infections in guinea pigs similar to that exhibited by the closely related phosphonoacetic acid (PAA); however, unlike PAA, PFA was not locally skin irritating. The therapeutic benefits of topical application of PFA were clearly evident when application was delayed for 48 h after virus inoculation, at which time lesions were well developed. The therapeutic effect was dependent on the concentration of PFA and the duration of treatment. PFA exhibited significant activity against established infections when administered intraperitoneally, although it was less effective via this systemic route than when applied topically.

A comparison of different antiviral drugs should be made preferably on an animal model in which infection closely resembles that in humans. Furthermore, to obtain a truly therapeutic situation, treatment should not begin until symptoms are evident. Cutaneous herpesvirus infection in guinea pigs has been investigated previously by Hubler et al. (5) and used by Schafer et al. (9) as a model for human infections. It has been established that the cumulative score and the time to healing are useful as variables in the determination of antiviral activity (S. Alenius and B. Öberg, Arch. Virol., in press). A comparison of the anti-herpesvirus drugs adenine arabinoside (ara-A), cytosine arabinoside (ara-C), 5'-iodo-2'-deoxyuridine (IUDR), ribavirin, and phosphonoacetic acid (PAA) on this model revealed that PAA was the only compound exhibiting good therapeutic activity when applied topically (Alenius and Öberg, in press). Also, PAA has been shown to be more active than ara-A, ara-C, IUDR, and ribavirin on cutaneous infection in hairless mice (3). A disadvantage observed with PAA was dermal toxicity at concentrations of 2% or more (3, 6; Alenius and Öberg, in press).

The trisodium salt of phosphonoformic acid (PFA; Fig. 1) is a new antiviral compound which inhibits cell-free herpesvirus DNA polymerase, prevents herpesvirus plaque formation in cell cultures (4, 8; E. Helgstrand, B. Eriksson, N. G. Johansson et al., Science, in press), and has a therapeutic activity against herpesvirus infection in guinea pigs and mice (Helgstrand et al., in press; E. R. Kern, J. C. Overall, Jr., L. A. Glasgow, J. M. Rero, and J. A. Boezi, Abstr. Annu. Meet. Am. Soc. Microbiol., 1978, S50, p. 221). We have made a quantitative comparison of the therapeutic activity of PFA and PAA (Fig. 1) at different concentrations on cutaneous herpesvirus infection in guinea pigs. To evaluate the therapeutic potential of PFA, we have varied the interval between inoculation and treatment, the concentration of the therapeutic agent, the number of treatments, and the route of administration.

MATERIALS AND METHODS

Cells and viruses. Baby hamster kidney cells were used to grow herpesvirus of low passage for inoculating guinea pigs. Herpes simplex virus type 1 (HSV-1) strain C42 was obtained from C. Kallander, and HSV-1 strain 90155 and HSV-2 strain 91075 were obtained from E. Lycke. Virus titration was done on African green monkey kidney cells according to the method of Ejercito et al. (2). At 500 μM PFA or PAA in the medium during plaque assay, the titers of the virus strains used were reduced by more than 99.9%.

Animals and inoculation procedure. Male, white, Dunkin-Hartley guinea pigs, 300 to 350 g, were inoculated on six sites on the back with a vaccination gun as described earlier (Alenius and Öberg, in press). A total of 90 animals were used in the study. A 20-μl portion of virus with a titer of 105 plaque-forming units per ml was applied to each skin area.

Treatment. Local treatment was given as topical applications of 30 μl at 12-h intervals. PFA and PAA were dissolved in water with 0.1% Tween 80 and 10% (wt/wt) glycerol and adjusted to pH 6.0. The six infected areas on each guinea pig were used in a cross-
wise fashion with three areas for vehicle (Tween-glycerol-water) and three areas for substance. The solutions were applied with a micropipette, spread over the infected sites, and allowed to dry on the skin before the animals were placed in cages. The local application of PAA has no effect on adjacent infected areas (Alenius and Öberg, in press) and this has also been observed for PFA. Intraperitoneal treatment was given as 2 ml of sterile solution at pH 7.2.

Scoring system. The score system has been described earlier (Alenius and Öberg, in press). The following scores were given for the appearance of inoculated skin: 0.5, erythematous and slightly edematous; 1, erythema and one or two small vesicles; 2, erythema and numerous small vesicles; 3, numerous large vesicles and, if in close juxtaposition, coalesced; III, vesicles dried, large crusts; II, crusts fallen off to ca. 50%; I, ca. 10% of the crusts remaining; and 0, uninfected or healed areas, no crusts or vesicles.

\[
\begin{align*}
\text{Na}_3^+ & \quad \text{PFA} \\
\text{Na}_3^+ & \quad \text{PAA}
\end{align*}
\]

FIG. 1. Structures of the trisodium salts of PFA and PAA.

Results

Therapeutic effects of PFA and PAA. A comparison of the effects of PFA and PAA at 100 mM on HSV-1 infections is shown in Fig. 2. The pattern of the curves indicates a very similar effect. A quantitative comparison between 5, 50 and 100 mM PFA and PAA was made by calculating the cumulative score for the treated areas from day 0 through day 14. The time to healing was also determined and the results are given in Table 1. No significant difference in the cumulative score or time to healing could be observed between PFA and PAA. The lowest possible cumulative score should be 2.5 since this score had already been reached when treatment started. A dermal toxicity was observed for 100 mM PAA on both days 5 and 12 after inoculation. A transient dermal toxicity was noted for 50 mM PAA in some experiments. No such effects were seen with PFA. A comparable effect on HSV-2 cutaneous infection was ob-
ALENIUS, DINTER, AND ÖBERG

**Table 1. Therapeutic effect of PFA and PAA**

<table>
<thead>
<tr>
<th>Substance</th>
<th>No. of skin areas</th>
<th>Cumulative score Mean &amp; SD</th>
<th>Time to healing (days) Mean &amp; SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mM</td>
<td>5</td>
<td>15.3 6.6</td>
<td>9.0 3.0</td>
</tr>
<tr>
<td>50 mM</td>
<td>5</td>
<td>7.3 2.6</td>
<td>6.0 1.6</td>
</tr>
<tr>
<td>100 mM</td>
<td>5</td>
<td>5.5 1.7</td>
<td>4.6 0.9</td>
</tr>
<tr>
<td>PAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mM</td>
<td>5</td>
<td>12.9 8.8</td>
<td>8.2 2.6</td>
</tr>
<tr>
<td>50 mM</td>
<td>5</td>
<td>6.3 1.9</td>
<td>5.2 1.1</td>
</tr>
<tr>
<td>100 mM</td>
<td>5</td>
<td>5.1 0.9</td>
<td>4.6 0.9</td>
</tr>
<tr>
<td>Solvent control</td>
<td>30</td>
<td>22.1 2.4</td>
<td>10.9 0.8</td>
</tr>
</tbody>
</table>

* Cumulative score and time to healing were determined after treatment as in Fig. 2. The arithmetic mean and the standard deviation (SD) are presented.

The healing was served for PFA and PAA at 5, 50, and 100 mM concentrations, and the other HSV strain tested (90155) was equally sensitive to PFA and PAA (data not shown).

No difference in therapeutic activity was observed when PFA was used at pH 4, 5, 6, or 7 in water solution with 0.1% Tween 80 and 10% (wt/wt) glycerol. Tween and glycerol were necessary to obtain satisfactory adhesion of the solution to the skin. PFA was also active in Monash solution (45% isopropanol-10% glycerol-45% water). Due to the insolubility of PFA in dimethyl sulfoxide, this was not a suitable solvent.

**Dose response determination for PFA.**

Treatment of HSV-1 infections was started 48 h after inoculation and continued for 4 days with 2 daily applications of 5, 10, 20, 50, and 100 mM PFA solution. This corresponds to 0.15, 0.30, 0.60, 1.5 and 3.0% PFA, respectively. Figure 3 shows percent reductions in cumulative scores for different concentrations. At 50 and 100 mM PFA good effects were obtained. The time to healing was considerably shortened at concentrations of 50 mM or higher (Table 1).

**Time dependence for treatment.**

The influence of varying the time between inoculation and initiation of treatment with PFA is shown in Fig. 4. Infected areas were treated with 2% PFA solution for 3 days starting at 4 h and 1, 2, 3, 4, and 5 days after inoculation. Control infections were treated with solvent. Early treatment was most effective in preventing the infection from reaching the highest score. The cumulative scores for treated areas were calculated and compared to those of the untreated areas. PFA had good therapeutic activity even when added 2 days after inoculation, but the effect decreased rapidly when treatment was delayed. There was also a corresponding reduction in time to healing. To obtain a 50% reduction in time to healing, it was necessary to initiate treatment no later than 48 h after inoculation.

**Number of treatments.**

The therapeutic effectiveness of 1, 2, 4, and 6 treatments with 2% PFA is compared in Fig. 5. The experiment shows that fewer than four treatments are not optimal and that six treatments with 2% PFA gave a similar reduction as eight treatments with 3% PFA (100 mM) (see Fig. 2). It is evident that fewer than four treatments had little effect on the time to healing. The cumulative score was slightly reduced even after one treatment with 2% PFA, and a good effect was observed after four treatments. No variation in cumulative score was seen when the solvent was used for one, two, four, or six applications.

**Intraperitoneal treatment with PFA.**

Intraperitoneal administration of PFA had a therapeutic effect on cutaneous HSV-1 infection as shown in Fig. 6. The slight reduction in score observed in Fig. 6, when 20 mg/kg was used, was significant (P < 0.05), and so was the reduction.
in time to healing ($P < 0.05$). At 100 mg/kg the effect was more pronounced, and a significant reduction in cumulative score ($P < 0.001$) and time to healing ($P < 0.001$) were obtained. The reduction in cumulative score was significantly better ($P < 0.05$) at 100 mg/kg than at 20 mg/kg.

The time from inoculation to when the vesicles started to dry and form crusts occurred between days 5 and 6 after intraperitoneal administration of PFA (Fig. 6). This usually occurs 1 day later, between days 6 and 7 in this animal model.

**DISCUSSION**

The guinea pig model for cutaneous herpesvirus infection (5) has been useful to evaluate antiviral drugs (1, 9; Alenius and Öberg, in press). This model was used to test the new...
antiviral compound PFA (4, 8; Helgstrand et al., in press) and to compare it to PAA, which has been reported to have a good topical activity against cutaneous HSV infections in two animal models (3; Alenius and Öberg, in press). The therapeutic effect of PFA on HSV-1 infections does not seem to have the side effect of dermal toxicity shown by PAA. A more thorough comparison of PFA and PAA in other animals is clearly indicated. It was recently reported that PFA was active in genital HSV-2 infections in mice and guinea pigs (Kern et al., Abstr. Annu. Meet. Am. Soc. Microbiol., 1978, S50, p. 221). An advantage to the use of PFA is its solubility in water in contrast to IUdR, which has to be used in dimethyl sulfoxide, or ara-A, which is sparsely soluble in water. Furthermore, IUdR and ara-A are not active in a therapeutic situation on the HSV guinea pig model (Alenius and Öberg, in press). In earlier reports on topical treatment of cutaneous herpesvirus infections treatment has started before clear symptoms have appeared (1, 3, 9).

Both PFA and PAA had good therapeutic activity when treatment was started after vesicles had appeared. No significant differences in cumulative score or time to healing on HSV-1 infection could be observed when the compounds were compared on an equimolar basis (Table 1; Fig. 2). This correlates with the similarity in inhibition of HSV-1 DNA polymerase and inhibition of HSV-1 plaque formation observed for PFA and PAA (8; Helgstrand et al., in press). At concentrations higher than 100 mM a comparison between PFA and PAA was difficult due to the dermal toxicity of PAA.

The lowest concentration of PAA used topically to obtain an effect on cutaneous infection in mice has been reported to be 5.5 mM (0.1%), and a good effect has been obtained with 55 mM (1%) PAA; however, the treatment was started before symptoms developed (3). These results correlate with the present findings that a good effect was obtained with 50 mM PAA or PAA both in cumulative score and in the time to healing. The dose-response curve (Fig. 2) showed that a maximal effect was obtained at 100 mM PAA when treatment was initiated 48 h postinoculation. It is not yet possible to correlate the inhibition of HSV DNA polymerase (4, 8; Helgstrand et al., in press) with the activity on cutaneous infection since the intracellular concentration of PFA is not known.

When contemplating use of an antiviral drug, it is of importance to consider how late during infection therapy may be initiated. PFA had a good effect (Fig. 4) on the cumulative score and the time to healing when applied 2 days after inoculation. If initiated 1 day after inoculation when only erythema can be seen, treatment with PFA prevents most of the vesicle formation. In the human herpes labialis infection, the time to healing is 8 to 10 days (11) compared with 11 days for the guinea pig cutaneous HSV infection used here (Alenius and Öberg, in press). The time to crust formation is 1.8 days from the first symptoms in human herpes labialis (11) and 6 to 7 days from inoculation in the guinea pig.
model. If the guinea pig and the human infections are analogous, a time comparison indicates that treatment of herpes labialis should be feasible as late as 1 to 2 days after the first symptoms.

The number of treatments has only been tested starting 48 h postinoculation with two daily applications; it was found that 6 applications were required for full activity (Fig. 5). It is quite likely that the number of applications necessary will depend on both the time of initiation of treatment and the concentration. The number of applications used for good activity on hairless mice has been reported to be 12 for PAA 1% (3) and 6 for 2% PAA (6).

The slight decrease in average score at low concentrations of PFA or few treatments was the result of a decrease in some areas and no effect in others (Table 1). As the concentration or the number of treatments increased, all areas responded to the treatment.

To reduce possible toxicity, a topical application of a drug on a cutaneous HSV infection is preferable; however, our data show that a systemic treatment also was possible starting 2 days postinoculation (Fig. 6). A dose of 100 mg of PFA per kg per day had a significant therapeutic activity when the drug was given intraperitoneally for 6 days. This corresponds to the data published for systemic treatment with PAA of cutaneous HSV infection in mice (3) where a good effect was observed at 200 mg/kg/day starting 1 day postinoculation and continuing for 6 days.

The therapeutic activity of PFA on infected guinea pig skin was also evident with HSV-2, and this indicates that PFA could be active in HSV-2 genital infections. The HSV-2 strains tested have resulted in paralysis (Alenius and Öberg, in press), a complication not observed with HSV-1, even though a therapeutic effect on the cutaneous infection was obtained. This paralysis could probably be avoided by administration of HSV hyperimmunserum (12). The effect of an earlier treatment with PFA or PAA on paralysis has not been investigated.

The good therapeutic activity of PFA and its apparent lack of dermal toxicity in guinea pigs makes it a potential candidate for use on cutaneous infections in humans. Recent results have shown PFA to be active against genital HSV-2 infections in guinea pigs and herpes keratitis in rabbits (S. Alenius, L. Berggren, U. Laurent, and B. Öberg, manuscripts in preparation).

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LITERATURE CITED