Chemotherapy of Genital Herpes Simplex Virus Type 2 Infections of Female Hamsters

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Received for publication 22 November 1976

The antiviral activity of 1-β-D-arabinofuranosylcytosine (ara-C, cytarabine, Cytosar), 5-iodo-2′-deoxyuridine (IdUrd), 9-β-D-arabinofuranosyladenine (ara-A), and disodium phosphonoacetate (PAA) have been compared in herpes simplex virus type 2 (HSV-2)-infected primary rabbit kidney cells and in female hamsters with genital HSV-2 infection. In vitro, ara-C and IdUrd were more active than ara-A, and PAA was least active. In female hamsters with genital HSV-2 infection, intravaginal treatment with PAA or ara-A was more effective than either ara-C or IdUrd. PAA was more active than ara-A when treatment was initiated early (1 h) after infection. The activity of PAA was greatly reduced if initiation of treatment was delayed for 24 h. Both PAA and ara-A reduced the virus titers of the vagina and protected hamsters from death when the drugs were given by either the intravaginal or subcutaneous route, with intravaginal treatment being more effective.

Genital herpesvirus infections, primarily caused by herpes simplex virus type 2 (HSV-2), are transmitted by venereal contact (9, 26). The infection can be painful and recurrent. If lesions are present late during the gestation period, the neonate is at high risk, resulting in disease with high morbidity and mortality (25). Furthermore, HSV-2 has been implicated in the etiology of cervical carcinoma (2, 4, 23).

At the present time, there is no effective treatment for genital HSV infections. However, several agents with antiherspes activity in other systems have been studied in herpes genitalis. Topical application of certain heterocyclic dyes, followed by exposure to light, has been proposed as a method for treating this disease (5). However, in subsequent studies with proflavine (34), or in a double-blind study with neutral red (24), it was not possible to demonstrate efficacy.

We have recently described the experimental genital infection of female hamsters with HSV-2 (30). In the studies described herein, several drugs with reported antiherspes activity have been compared in vitro and in female hamsters with genital HSV-2 infection.

MATERIALS AND METHODS

Virus. Stocks of HSV-2 (strain 35D) were supplied by L. T. Chien, University of Tennessee. The virus had a titer of 7.5 × 10⁶ to 1.0 × 10⁷ plaque-forming units per 0.5 ml on primary rabbit kidney monolayers.

In vitro antiviral activity. Contiguous monolayers of primary rabbit kidney cells were infected by incubating 0.5 ml of stock virus with drained monolayers (60-mm plastic petri dishes, Falcon) for 60 min at 37°C. The monolayers were washed and refed with 4.5 ml of Eagle medium containing 3% fetal bovine serum. The drugs were prepared in Hanks solution, and 0.5 ml of the appropriate dilution was added to each plate, resulting in the concentrations indicated. The cultures were harvested at 70°C when approximately 50 to 75% of the cells in the culture showed evidence of cytopathology. The virus titers of the frozen-thawed cultures were determined by the plaque method on rabbit kidney monolayers.

Genital infection of female hamsters. The method of infecting hamsters with HSV-2 has been described (30). Briefly, female hamsters obtained from Charles River Lakeview (Newfield, N.J.) were housed in stainless-steel cages in a room containing male hamsters. The hamsters were infected within 1 week of arrival and at that time weighed 45 to 50 g. The vaginas were washed with 2 ml of saline about 1 h before inoculation of 0.1 ml of HSV-2 intravaginally.

Drug treatment. The drugs were obtained from commercial sources, except for disodium phosphonoacetate (PAA), which was generously provided by H. I. Skulinck of these laboratories. For the in vivo studies, the drugs were prepared in saline (0.9% NaCl) solution. For vaginal therapy, 0.1 ml of drug was delivered intravaginally using a tuberculin syringe fitted with a Temflex no. 24 vinyl-covered 22-gauge needle. Drugs were given three times each day at 8 a.m., noon, and 4 p.m. The drug concentration was 20 mg/ml.

For systemic treatment, the drugs were prepared at the appropriate concentration in saline solution and given by subcutaneous injection. Drug treat-
ments were given twice each day (8 a.m. and 4 p.m.).

Fifteen hamsters comprised each group.

Vaginal swabs. Cotton-tipped swabs on 1/8-inch (ca. 0.3 cm) wooden dowels, wetted with Hanks solution, were used to swab the vaginas. The swabs were placed in tubes containing 2 ml of Eagle medium-3% fetal bovine serum. The samples were stored at -40°C until the time of virus assay by the plaque method on primary rabbit kidney cells.

RESULTS

Figure 1 compares the HSV-2 yields from primary rabbit kidney monolayers treated with different concentrations of 1-β-p-arabinofuranosylcytosine (ara-C), 5-iodo-2′-deoxyuridine (IdUrd), 9-β-p-arabinofuranosyladenine (ara-A), and PAA. These data show that ara-C was more active than the other compounds included in this study, and that PAA was the least inhibitory. None of the compounds was cytotoxic under these conditions. The effect of treating hamsters infected vaginally with HSV-2 by the topical application of drug is shown in Fig. 2. In this study, 13 of 15 hamsters treated with saline died (mean survival time [MST], 9.6 days). ara-C and IdUrd had little effect on the disease process. In both cases, 80% of the animals died and the MST was 10.9 and 11.5 days, respectively. ara-A treatment extended the MST to 15.6 days, but only 4 of the 15 hamsters survived the infection. PAA was most effective of the agents tested, in that 80% of the hamsters were protected with this drug. When the drug solutions were adjusted to pH 7, the resulting antiviral activity was nearly the same.

Vaginal swabs were taken from those animals depicted in Fig. 2 at 120 h after virus inoculation, i.e., 16 h after the final drug treatment. Titration of these samples (Table 1) showed that both ara-C and ara-A treatment reduced the virus titers, compared with the saline-treated animals, by nearly 1 log. IdUrd was less effective. PAA was the most effective drug in this series in that virus could not be recovered from any of the vaginal swabs taken at this time. Even though the virus titers of the ara-A-treated hamsters were similar to those of the ara-C-treated group, ara-A treatment was considerably more beneficial in delaying death than was either ara-C or IdUrd (Fig. 2).

Figure 3 compares the activity of PAA and ara-A when therapy was initiated at different times after virus inoculation. In this study, drug was administered for 4 days, rather than the 5 days described in Fig. 2. When treatment
was initiated on day 0 (1 h after virus inoculation), ara-A treatment resulted in 33% survivors with an MST of 16 days, whereas PAA treatment resulted in 60% survivors with an MST of 17.3 days (Fig. 3A). This should be compared with saline treatment after which none of the hamsters survived and the MST was 7.9 days. If treatment was delayed for 24 h after inoculation (Fig. 3B), ara-A increased the percentage of hamsters surviving from 13.3% for the saline-treated controls to 40%, and the MST was increased from 9.3 days for the salinetreated controls to 18.3 days. PAA was less active when treatment was delayed; only 13% of the hamsters survived and the MST was 11 days, or about 2 days longer than that for saline-treated groups.

The virus titers of the vaginal swabs from 10 hamsters in each group taken 16 h after the final drug treatment (Table 2) showed that ara-A reduced the virus titers but not the number of animals shedding virus, compared with the saline-treated group, whether the therapy was initiated 1 or 24 h after virus inoculation. The difference in the titers between the ara-A- and saline-treated groups were 1.4 and 1.25 logs when therapy was initiated 1 or 24 h after virus inoculation, respectively. When PAA treatment was initiated at 1 h, virus was recovered from only one of 10 hamsters. Delaying PAA treatment until 24 h after virus inoculation greatly reduced the antiviral activity; seven of nine hamsters shed virus, and the mean virus titer was about 1 log less than that for the saline-treated group: this difference was not significant.

Table 1. Effect of intravaginal drug treatment on vaginal HSV-2 virus titers at 120 h after virus inoculation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nv/Nt*</th>
<th>Mean*</th>
<th>S.D.*</th>
<th>ΔMean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>9/12a</td>
<td>2.37</td>
<td>1.56</td>
<td></td>
</tr>
<tr>
<td>Drug (20 mg/ml) ara-C</td>
<td>8/15</td>
<td>1.39</td>
<td>1.58</td>
<td>-0.98**</td>
</tr>
<tr>
<td>ara-A</td>
<td>8/15</td>
<td>1.41</td>
<td>1.52</td>
<td>-0.96*</td>
</tr>
<tr>
<td>IdUrd</td>
<td>10/15</td>
<td>1.83</td>
<td>1.55</td>
<td>-0.54 (NS)</td>
</tr>
<tr>
<td>PAA</td>
<td>0/15</td>
<td>0</td>
<td></td>
<td>-2.37**</td>
</tr>
</tbody>
</table>

* Nv refers to the number of hamsters shedding virus, and Nt is the total number of hamsters in the group.
* Mean indicates the log of the mean of the virus titers.
* S.D., Standard deviation.
* Three hamsters were dead.
* ** indicates P < 0.05, ** indicates P < 0.01, and NS is nonsignificant.

For example, vaginal treatment with ara-A increased the number of survivors from 6.7% for the saline-treated group to 53.3%. The MSTs were 9.8 and 18.1 days, respectively, for saline and ara-A. Vaginal treatment with PAA was most effective in that 93% of the hamsters survived.

When the drugs were given subcutaneously, 33% of the hamsters survived with ara-A (250 mg/kg) treatment, and the MST was 16.8 days. Treatment with PAA (400 mg/kg) by the same route resulted in 46.7% survivors, with an MST of about 16 days, whereas 13.3% of the salinetreated animals survived, and the MST was 10.5 days. The ara-A and PAA concentrations used for subcutaneous treatment were previously shown to be effective in HSV-infected hamsters (32) and mice (18, 31), respectively.

The course of the vaginal infection in hamsters treated vaginally or subcutaneously with ara-A or PAA (Fig. 4) was followed by daily virus titrations of vaginal swabs from 10 hamsters.
TABLE 2. Vaginal HSV-2 virus titers from hamsters treated with saline, ara-A, or PAA according to the schedule in Fig. 3a

<table>
<thead>
<tr>
<th>Determination</th>
<th>Treatment (day 0)</th>
<th>Treatment (day 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>ara-A</td>
</tr>
<tr>
<td>Nv/Nt</td>
<td>9/10</td>
<td>8/10</td>
</tr>
<tr>
<td>Mean</td>
<td>2.45</td>
<td>1.07</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.45</td>
<td>0.89</td>
</tr>
<tr>
<td>ΔMean</td>
<td>-1.39**</td>
<td>-2.40**</td>
</tr>
<tr>
<td>Survivors</td>
<td>0/15</td>
<td>5/15 (33%)</td>
</tr>
<tr>
<td>MST</td>
<td>7.9</td>
<td>16</td>
</tr>
</tbody>
</table>

* Vaginal swabs were taken 16 h after the final drug treatment. See Table 1 for definitions of symbols.

Virus titers were significantly less than saline with the ara-A treatment. Vaginal treatment with PAA resulted in nearly complete protection from HSV infection, as determined by the vaginal swab virus titers.

When ara-A was administered subcutaneously, the vaginal virus titers were significantly less than the control, except for the 96-h samples (Table 3). The number of hamsters shedding virus at 120 and 144 h was decreased. Subcutaneous treatment with PAA decreased the number of hamsters shedding virus from 6 of 10 at 24 h to 1 of 10 at 72 h. At all times studied, the virus titers were significantly less than in the saline-treated group. In spite of the reduction in the number of hamsters shedding virus during this period of observation, nearly half of the animals died during the following 2 weeks.

DISCUSSION

The compounds included in the present study were selected because of demonstrated antiviral activity for HSV in vitro and in animals by the topical application directly to the site of the herpesvirus infection.

The relative in vitro antiviral activity for HSV-1 and HSV-2 for some of the compounds studied herein has been somewhat controversial. For example, ara-A was found to be more inhibitory than IdUrd for HSV-2 in chicken embryo cells and, further, it was observed that HSV-1 was more susceptible to either drug than HSV-2 (20). Person et al. (29) showed that the antiviral activity of ara-A and IdUrd for HSV-2 was dependent upon the host cell; e.g., HSV-2 was considerably less susceptible to IdUrd in chicken embryo cells than in HeLa cells. Fiala et al. (6) showed that ara-C was more active than ara-A, and IdUrd was less active for several strains of HSV-2 in WI-38 cells. These studies were extended by Marks (22), who showed that IdUrd was somewhat less...
active than ara-C, but both compounds were more active than ara-A in primary cultures from rabbit kidney or rat brain infected with several isolates of HSV-1 and HSV-2. The results presented herein agree with the latter studies, indicating that ara-C is the most active compound, followed by IdUrd and ara-A. PAA was the least active compound in the in vitro studies.

When these same drugs were applied topically to female hamsters with genital HSV-2 infection, PAA was the most active compound; ara-A was less active than PAA but more active than either ara-C or IdUrd. Treatment with ara-C and IdUrd did not increase the number of survivors and only slightly extended the MST. Hamsters treated with ara-C yielded less virus from vaginal swabs than did those treated with IdUrd, but neither drug significantly reduced the number of hamsters yielding virus. ara-A treatment extended the MST and increased the number of hamsters that survived infection. Approximately 50 to 80% of the hamsters treated with ara-A yielded virus, and the virus titers were reduced. PAA treatment, when drug therapy was initiated 1 h after virus inoculation, resulted in 80 to 93% survivors. Treatment with PAA under these conditions was highly effective in reducing the number of hamsters from which virus could be recovered in the vaginal swabs. In the above studies, the drugs were prepared in saline, and other vehicles were not used.

A topical antiherpetic activity of IdUrd was first described by Kaufman, who used rabbits with herpes keratitis (12), and later in clinical keratitis (15). In addition, it has been shown that IdUrd in dimethyl sulfoxide is effective when applied topically to HSV infections of guinea pig skin (35) and in man (21). ara-C is topically active against experimental herpes keratitis in rabbits (36) and in man (14); however, ara-C is not used in clinical keratitis because of its corneal toxicity (13).

Sloan (32) has reviewed the antiviral activity of ara-A in animals with experimental herpesvirus infections, and Hyndiuk and Kaufman (10) have shown that ara-A is effective against ocular herpes simplex infections. It has recently been shown that topical application of ara-A was effective in treating herpetic eye disease in patients intolerant of or resistant to IdUrd (28). Systemic administration of ara-A to patients with cutaneous HSV-1 infections resulted in dramatic improvement, whereas the effect on HSV-2 lesions was less dramatic (3). ara-A has also been shown to be effective in immunosuppressed patients with varicella zoster (1, 11, 37). However, in a double-blind study comprised of 32 men with virologically proven herpes genitalis, topical ara-A (3% in petrolatum vehicle) was not effective in altering the quantitative virology or the clinical course of the disease (8). ara-A, given intraperitoneally at 500 mg/kg to newborn mice inoculated intranasally with HSV-2, suppressed virus replication for 2 days and increased the MST by 2 days but did not increase the number of mice surviving the infection (16). Subcutaneous treatment with ara-A protected mice with

<p>| Table 3. Vaginal HSV-2 titers from hamsters treated intravaginally or subcutaneously with saline, ara-A, or PAA* |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>h postinfection</strong></th>
<th><strong>Saline Nv/NT</strong></th>
<th><strong>Mean ± S.D.</strong></th>
<th><strong>ara-A Nv/NT</strong></th>
<th><strong>Mean ± S.D.</strong></th>
<th><strong>PAA Nv/NT</strong></th>
<th><strong>Mean ± S.D.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intravaginal treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>10/10</td>
<td>2.66 ± 0.92</td>
<td>6/10</td>
<td>1.08 ± 1.05</td>
<td>0/10</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>48</td>
<td>10/10</td>
<td>3.46 ± 0.80</td>
<td>6/10</td>
<td>1.39 ± 1.53</td>
<td>0/10</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>72</td>
<td>10/10</td>
<td>2.56 ± 1.04</td>
<td>6/10</td>
<td>1.88 ± 1.61</td>
<td>1/10</td>
<td>0.03 ± 0.07</td>
</tr>
<tr>
<td>96</td>
<td>10/10</td>
<td>3.36 ± 0.78</td>
<td>6/10</td>
<td>1.87 ± 1.59</td>
<td>0/10</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>120</td>
<td>9/10</td>
<td>2.64 ± 1.12</td>
<td>6/10</td>
<td>1.19 ± 1.03</td>
<td>0/10</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>144</td>
<td>7/8*</td>
<td>2.08 ± 1.15</td>
<td>6/10</td>
<td>1.00 ± 1.02</td>
<td>0/10</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td><strong>Subcutaneous treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>10/10</td>
<td>3.07 ± 0.95</td>
<td>7/10</td>
<td>1.52 ± 1.24</td>
<td>6/10</td>
<td>1.26 ± 1.14</td>
</tr>
<tr>
<td>48</td>
<td>10/10</td>
<td>3.68 ± 0.57</td>
<td>8/10</td>
<td>1.71 ± 1.16</td>
<td>4/10</td>
<td>0.83 ± 0.89</td>
</tr>
<tr>
<td>72</td>
<td>10/10</td>
<td>2.54 ± 0.81</td>
<td>7/10</td>
<td>1.68 ± 1.35</td>
<td>1/10</td>
<td>0.33 ± 0.93</td>
</tr>
<tr>
<td>96</td>
<td>9/10</td>
<td>2.44 ± 1.51</td>
<td>7/10</td>
<td>1.69 ± 1.40</td>
<td>1/10</td>
<td>0.36 ± 1.14</td>
</tr>
<tr>
<td>120</td>
<td>10/10</td>
<td>2.91 ± 0.93</td>
<td>4/10</td>
<td>0.69 ± 0.97</td>
<td>1/10</td>
<td>0.27 ± 0.87</td>
</tr>
<tr>
<td>144</td>
<td>9/9*</td>
<td>2.33 ± 0.94</td>
<td>1/10</td>
<td>0.05 ± 0.15</td>
<td>2/10</td>
<td>0.54 ± 1.28</td>
</tr>
</tbody>
</table>

* Ten hamsters comprised each group. See Table 1 for definitions of symbols.
* Two dead.
* One dead.
HSV-1 or HSV-2 encephalitis (33). ara-A has been found to protect mice from cutaneous HSV infection if the drug is given intradermally at the site of infection (19) or intraperitoneally (17). ara-A treatment intraperitoneally of adult female mice with genital HSV-2 infection resulted in an extension of the MST without affecting the vaginal virus titers or the final mortality (27). Subsequently, it was observed that intravaginal ara-A treatment of mice with vaginal HSV-2 infection did not alter the virus replication in the vagina, nor did ara-A decrease the final mortality, whereas PAA was highly effective if given early after infection (E. R. Kern, J. C. Overall, and L. A. Glasgow, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D. C., Abstr. 239, 1975). The conflict concerning the reported efficacy of ara-A in mice with vaginal HSV-2 infections and the present studies with vaginally infected hamsters may be due to differences in the susceptibility of HSV-2 used for inoculation or the disposition of ara-A in the two animal species.

PAA was originally shown by Shipkowitz et al. (31) to be active when applied topically or given orally to mice with cutaneous HSV-2 infection. Subsequently, it was shown that intraperitoneal treatment was effective in mice with cutaneous HSV-1 infections (18). PAA was also effective when administered as drops to the eyes of rabbits with experimental keratitis (7) and when applied topically to the skin of mice with cutaneous HSV-1 lesions (38). Of interest is the observation that PAA, in the latter study, was effective only if treatment was initiated before 24 h after virus inoculation (38). PAA was similarly effective in mice inoculated intraperitoneally or vaginally, but only when drug treatment, given by the systemic or topical route, was initiated within 24 h after inoculation (E. R. Kern, J. C. Overall, and L. A. Glasgow, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D. C., Abstr. 239, 1975). In the studies reported herein, it was also observed that PAA was highly effective (using vaginal virus titers or mortality as criteria for antiviral activity) if topical drug treatments were initiated on the day of infection, but not if therapy was delayed for 1 day. ara-A was nearly as effective whether treatment was initiated on the day of infection or 24 h later.

In the present studies, no correlation existed between the in vitro antiviral activity and the activity resulting from the intravaginal treatment of female hamsters with genital HSV-2 infection. In fact, an inverse relationship existed: ara-C and IdUrd, the most inhibitory compounds in vitro, were the least active in hamsters, and ara-A and PAA, the least active in vitro, were the most active in hamsters. Both ara-A and PAA were effective in reducing vaginal virus titers and protecting hamsters from death when given parenterally (subcutaneously), as well as intravaginally, in this model. PAA appeared to be more active when given intravaginally. However, as in the other model infections (38; E. R. Kern, J. C. Overall, and L. A. Glasgow, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D. C., Abstr. 239, 1975) PAA was maximally active when drug treatment was initiated early after infection. ara-A retained activity even when therapy was initiated 24 h after virus inoculation.

ACKNOWLEDGMENTS

I wish to acknowledge the technical assistance of Barbara A. Court, E. B. Sweeney, and P. T. Maida, as well as P. Good for the statistical analysis of the data.

LITERATURE CITED


