Therapy of Experimental Herpes Simplex Encephalitis with Aciclovir in Mice

NO-HEE PARK,1, 2 DEBORAH PAVAN-LANGSTON,1, 3* SANDRA L. MCLEAN,1 AND DANIEL M. ALBERT2, 3

Eye Research Institute of the Retinal Foundation,1 Massachusetts Eye and Ear Infirmary,2 and Harvard Medical School,3 Boston, Massachusetts 02114

Received for publication 19 March 1979

This report is concerned with the capacities of aciclovir to protect mice challenged intracerebrally with multiple lethal doses of type 1 herpes simplex virus and to control multiplication of this virus in the brain. With treatment initiated 12 h after inoculation and continued for 4 consecutive days, aciclovir administered subcutaneously in daily doses ranging from 40 to 100 mg/kg led to 21-day survival rates of from 33 to 73% and reduced virus titers by 1 to 10−4 logs on postchallenge day 8. The therapeutic accomplishments of the 100-mg/kg doses of aciclovir were comparable to those of 1,000-mg/kg doses of vidarabine (9-β-D-arabinofuranosyladenine); however, as measured by impact on body weight, aciclovir was better tolerated than vidarabine at these similarly effective doses.

Aciclovir (ACV; 9-(2-hydroxyethoxymethyl)guanine, or acycloguanosine) has been introduced recently as a potent and selective antiviral agent against herpes simplex virus type 1 (HSV-1) and HSV-2 (6, 7, 9, 13, 16). In vivo this compound also inhibits the multiplication of varicella zoster virus, cytomegalovirus, and B virus but has no effect on vaccinia virus, adenovirus type 5, and a range of ribonucleic acid viruses (18). ACV penetrates infected cells to a greater degree than uninfected cells (6). Once introduced into cells, it is converted to a monophosphate form by an HSV-specific thymidine kinase and eventually to a triphosphate form. ACV triphosphate then acts by inhibition of viral DNA polymerase. An additional selective advantage is that ACV triphosphate has a 30-fold-greater affinity for viral deoxyribonucleic acid polymerase than for cellular deoxyribonucleic acid polymerase in vitro (6, 7). In the present study we evaluated the therapeutic efficacy of ACV for the herpes encephalitis by observing the capacities of ACV to protect mice inoculated intracerebrally with HSV-1 and to control the multiplication of this virus in the brain. Moreover, mice were weighed during and after the treatment period to analyze drug toxicity as manifested by changes in body weight in comparison with controls.

MATERIALS AND METHODS

Virus. HSV-1 McKrae strain was propagated on primary rabbit kidney cells to yield a titer of 2.6 x 107 plaque-forming units per ml. This virus stock was stored at −75°C until used in the current experiments.

Mice. Outbred albino male mice (CD-1 strain; purchased from Charles River Breeding Laboratories, Boston, Mass.) were maintained for 1 week in our animal laboratory. They were housed 10 to a cage and fed high-energy, ultradigestible Mouse Chow (Ralston Purina Co., St. Louis, Mo.). The average weight of mice was 20 ± 1 g when the experiments were initiated.

Compounds. The sodium salt of ACV was obtained through the courtesy of Burroughs Wellcome Co., Research Triangle Park, N.C. A vidarabine (9-β-D-arabinofuranosyladenine [ara-A]) suspension was kindly supplied by Warner Lambert–Parks, Davis & Co., Inc., Ann Arbor, Mich. Appropriate concentrations of ACV solution were prepared in a sterile physiological saline solution.

Inoculation of HSV-1 or saline. Mice anesthetized with sodium pentobarbital were inoculated intracerebrally with 10 50% lethal doses of HSV-1 stock (5.2 x 106 plaque-forming units). Control mice were injected intracerebrally with 20 μl of Dulbecco phosphate-buffered saline.

Survival studies. Ninety mice inoculated with HSV-1 were divided into six equal subgroups; the treatments of the subgroups were as follows: (i) phosphate-buffered saline, 0.2 ml/day; (ii) ACV, 40 mg/kg per day; (iii) ACV, 60 mg/kg per day; (iv) ACV, 80 mg/kg per day; (v) ACV, 100 mg/kg per day; and (vi) ara-A, 1,000 mg/kg per day.

Forty-five mice inoculated with saline were divided into three equal subgroups which were treated as follows: (i) phosphate-buffered saline, 0.2 ml/day; (ii) ACV, 100 mg/kg per day; or (iii) ara-A, 1,000 mg/kg per day.

Treatments were initiated 12 h after the inoculation of HSV-1 or saline and consisted of subcutaneous injections of two divided doses per day for 4 days. The mice were weighed on days 0, 2, 4, 8, 10, 14, and 16 after the inoculation to monitor weight change. The mice were also observed daily for 21 days to document
the survival rates and the mean survival time of mice that died. The brains of dead mice were removed and homogenized. The homogenates were centrifuged (2,000 rpm), the supernatant was inoculated onto green monkey kidney cell (CV-1; American Tissue Culture Collection, Rockville, Md.) monolayers, and the cytopathic effects were observed to confirm that the mice died of encephalitis.

**Determination of the viral titers in brains.** A total of 150 mice inoculated with HSV-1 were divided into six equal subgroups and treated similarly to those in the survival studies. Five animals from each subgroup were sacrificed on days 2, 4, 6, and 8 post-inoculation. Mice dying during these periods were excluded from the experiment. The brains of live mice were aseptically removed, weighed, pooled, and washed in sterile normal saline. After removal of the surface membranes, they were finely minced with scissors, homogenized, and made into a 10% suspension with sterile Hanks balanced salt solution. The suspension was frozen and thawed three times and centrifuged at 2,000 rpm for 5 min at 4°C. The supernatant was then serially diluted and assayed on green monkey kidney CV-1 monolayers by using an ordinary plaque technique (14).

**RESULTS**

**Survival rate and mean survival time.** Within 12 days post-inoculation, all infected saline-treated mice were dead. The mean survival time was 6.9 days. ACV at doses of 100, 80, 60 and 40 mg/kg per day for 4 days yielded survival rates of 73, 60, 33, and 33%, respectively. The mean survival time of mice that died was also significantly increased by 100 and 80 mg of ACV per kg per day (to 13.5 and 11.2 days, respectively) compared with saline-treated controls (100% dead at 6.9 days). Administration of ara-A also significantly increased the survival rate (80%) and mean survival time of mice that died (14.0 days), compared with the same control group. When ACV (100 mg/kg per day) or ara-A (1,000 mg/kg per day) was given to the saline-inoculated mice for 4 days, no deaths occurred (Table 1).

**Concentration of virus in brains.** Treatment with ACV or ara-A resulted in a notable reduction in the infectious virus from pooled brains (Fig. 1). ACV at a daily dose of 40 or 60 mg/kg reduced the virus titers 11- to 20-fold compared with the saline-treated controls during the period from day 2 to day 8 post-inoculation. Higher doses of ACV (80 and 100 mg/kg) and vidarabine gradually decreased the level of virus in brains and resulted in virus titers approximately ½ x 4 to 5 logs less than the titers in the saline-treated control brains on day 8 post-inoculation.

**Body weight of mice.** The comparative effects of ACV and ara-A on the body weight of mice are indicated in Table 2. ACV treatments of virus-inoculated mice resulted in a slight reduction of body weight by day 8 post-inoculation. However, the weight was recovered, and ACV-treated mice started to gain weight from day 10 post-inoculation. When the highest doses of ACV (100 mg/kg) was given to saline-inoculated mice, the growth was initially slower than that of the normal mice (saline-treated and saline-inoculated), but there was no significant difference in weight between these two groups from day 10 of the study (P < 0.2). Vidarabine treatments in virus- or saline-inoculated mice, at a dose of 1,000 mg/kg, led to a gradual decrease in weight by day 8 post-inoculation. Furthermore, the vidarabine-treated animals had not:

**Table 1. Effect of ACV on the survival rate of mice and mean survival time of mice that died.**

<table>
<thead>
<tr>
<th>Treatment group*</th>
<th>Inoculation with:</th>
<th>Survival at 21 days (no. surviving/no. tested)</th>
<th>Mean survival time (days) of mice that died</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiological saline, 0.2 ml/day</td>
<td>HSV-1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0/15</td>
<td>6.9 ± 0.65 (3-10)*</td>
</tr>
<tr>
<td>ACV, 40 mg/kg per day</td>
<td>Saline&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15/15</td>
<td>8.6 ± 1.25 (5-13)</td>
</tr>
<tr>
<td>ACV, 60 mg/kg per day</td>
<td>HSV-1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5/15&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9.4 ± 1.51 (5-13)</td>
</tr>
<tr>
<td>ACV, 80 mg/kg per day</td>
<td>HSV-1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5/15&lt;sup&gt;f&lt;/sup&gt;</td>
<td>11.2 ± 1.33 (7-14)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACV, 100 mg/kg per day</td>
<td>HSV-1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11/15&lt;sup&gt;f&lt;/sup&gt;</td>
<td>13.5 ± 0.50 (13-15)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>ara-A, 1000 mg/kg per day</td>
<td>Saline&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15/15</td>
<td>14.0 ± 0.58 (13-15)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
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* Treatments were initiated 12 h after the inoculation of 10 50% lethal doses of HSV-1 and consisted of subcutaneous injections given twice daily for 4 days.
<sup>d</sup> Ten 50% lethal doses of HSV-1 was inoculated by intracerebral injection.
<sup>f</sup> Numbers in parentheses are ranges.
<sup>e</sup> A 20-μl amount of Dulbecco phosphate-buffered saline was injected intracerebrally.
<sup>s</sup> Significantly different (P < 0.05) from the physiological saline-treated mice which were inoculated with HSV-1 (Fisher exact test, double tail).
<sup>g</sup> Significantly different (P < 0.05) from the physiological saline-treated mice (Student’s t test).
returned to their original weight by day 16 post-inoculation.

**DISCUSSION**

HSV encephalitis is one of the most frequent epidemic, life-threatening viral infections of the brain in the United States today (11, 12). Primary attack of HSV, both HSV-1 and HSV-2, is the main cause of herpes encephalitis (10, 12). However, a latent infection of HSV can possibly induce herpetic involvement of the central nervous system (10).

Recently, considerable effort has been dedicated to treating HSV encephalitis. Systemic therapy with iododeoxyuridine for the treatment of HSV encephalitis was initially met with some enthusiasm (3, 11). Two placebo-controlled, double-blind studies designed to evaluate the chemotherapeutic efficacy of iododeoxyuridine in HSV encephalitis were, however, prematurely terminated; iododeoxyuridine not only failed to prevent morbidity and mortality, but also caused severe toxicity, namely myelosuppression (1). Vidarabine has been shown to be an effective agent against HSV encephalitis in hamsters (17), mice (8, 18, 19), and humans (2, 20, 21). A pilot study by Ch’ien et al. (2) indicated a therapeutic effect of vidarabine in neonatal herpes encephalitis if it was administered early, within 3 days after the onset of neurological signs. Taber et al. (20) and Whiteley et al. (21) have, furthermore, reported the successful control of biopsy-proven HSV encephalitis with systemic vidarabine treatment. Although no serious acute toxicity has been reported in humans, anorexia, nausea and vomiting, weight loss, weakness, megaloblastic changes in erythroid elements of bone marrow, tremors, and thrombophlebitis have been encountered with systemic application of vidarabine in daily doses of 20 mg/kg (15). Tri-fluorothymidine (5-trifluoromethyl-2'-deoxyuridine) (4) and 5-ethyl-2'-deoxyuridine (5) were also introduced as effective agents for the management of HSV encephalitis in mice.

ACV has recently emerged as an effective and selective inhibitor of HSV replication. Schaeffer et al. (16) showed that ACV in ointment concentrations of from 0.3 to 3% was effective against established HSV epithelial keratitis in rabbits and was well tolerated. We have recently reported a controlled study of the activities of 3% ACV, 0.5% iododeoxyuridine, and 3% vidarabine ointments against an experimental HSV keratitis in rabbits. ACV was significantly more active than iododeoxyuridine and vidarabine, producing more rapid healing and no significant toxicity (13). Kaufman et al. (9) reported that ACV administered parenterally was effective in treating herpetic iritis in rabbits and prevented death from encephalitis.

A pilot study by Schaeffer et al. (16) indicated that ACV, administered orally in a dose of 100 mg/kg twice daily for 5 days, significantly increased the survival rate and mean survival time of mice inoculated intracerebrally with HSV-1. Our present experiments were designed to evaluate the chemotherapeutic efficacy of ACV against experimental HSV-1 encephalitis in mice, with responses measured in terms of survival rate, mean survival time, and virus titer in the brain. In addition, the efficacies of various nontoxic doses of ACV were compared with the effectiveness of the maximum tolerated dose of ara-A, a compound recently approved by the Food and Drug Administration for use in herpetic infections, including human herpetic encephalitis. To determine the nontoxic level of ACV, various doses of the agent were administered daily for 4 days to 20-g mice by two divided doses through subcutaneous injection. Daily doses of ACV up to 100 mg/kg for 4 days did not induce weight loss or other untoward reactions. Weight loss was significant at daily doses of 200 mg/kg for 4 days. The latter dose also effects a moderate reduction in number of Jerne hemo-lysin plaques (R. E. Keeney, Burroughs Well-
<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Inoculation with</th>
<th>Mean wt change (g) on post-inoculation days:</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Physiological saline, 0.2 ml/day</td>
<td>HSV-1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.3 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Saline&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.2 ± 0.06</td>
</tr>
<tr>
<td>ACV, 40 mg/kg per day</td>
<td>HSV-1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.7 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>Saline&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-0.5 ± 0.08</td>
</tr>
<tr>
<td>ACV, 60 mg/kg per day</td>
<td>HSV-1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.9 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Saline&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-0.9 ± 0.10</td>
</tr>
<tr>
<td>ACV, 100 mg/kg per day</td>
<td>HSV-1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.2 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Saline&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-0.5 ± 0.02</td>
</tr>
</tbody>
</table>

<sup>a,b,d</sup> See Table 1, footnotes a, b, and d, respectively.
<sup>*</sup> Numbers in parentheses indicate numbers of mice weighed.
come Co., personal communication). Based on these considerations, we used in the current study doses of 40, 60, 80, and 100 mg/kg per day. In vitro antiviral activity of ACV was not determined. However, ACV has been reported to be 160 times more active than ara-A against HSV-1 in plaque reduction assays (16). Doses of ara-A up to 1,000 mg/kg per day for 4 days were tolerated by mice. A dose of 2,000 mg/kg per day was lethal to more than 50% of the animals. Therefore, ara-A was used in a dose 1,000 mg/kg per day in the current studies. This dosage has been shown to provide a 100% protection of weanling mice challenged intracerebrally with 10 50% lethal doses of HSV-1 (8).

The present studies have shown that ACV is highly effective against HSV-1 encephalitis in mice and that this activity, measured by increased survival rates or mean survival times, is dose dependent. ACV also diminished brain titers of HSV-1 in infected mice in a dose-dependent manner. Ara-A significantly increased survival rate and mean survival time and decreased brain virus titers in infected mice. However, ara-A, unlike ACV, caused a significant decrease in body weight in uninfected, saline-treated mice. Thus, ACV appears to be capable of fulfilling several important criteria for an effective antiviral agent: increased survival rate and survival time at an apparently nontoxic dose. On the basis of these observations, we believe that ACV clearly merits further investigation as a highly effective antiviral agent, not only for the treatment of herpes encephalitis but also as therapy for other manifestations of herpetic disease.

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service research grants EYNS-02268 and EY-00208 from the National Institutes of Health.

LITERATURE CITED