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Acyclovir treatment of disseminated herpes simplex virus type 2 infection in weanling mice: alteration of mortality and pathogenesis

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Summary

Intranasal inoculation of weanling mice with herpes simplex virus type 2 (HSV-2) provides an experimental infection that closely resembles disseminated and central nervous system HSV infections of human neonates. Intraperitoneal treatment with acyclovir (ACV) successfully reduced mortality even when therapy was begun as late as 2 days and oral therapy as late as 4 days after viral challenge. Treatment with ACV beginning on day 1 completely inhibited HSV-2 replication in lung, spleen, kidney, olfactory lobe, and cerebrum and decreased viral titers in the pons by 2–3 logs. Comparison of these data with our previous experiments using adenine arabinoside and adenine arabinoside 5′ monophosphate indicates that ACV is more effective in the murine model of neonatal disease and suggests that ACV may also be more effective in treating the disease in humans.

herpes simplex virus type 2; neonatal herpes; mice; acyclovir; pathogenesis; encephalitis

Introduction

Herpes simplex virus (HSV) is the causative agent of an uncommon but very severe infection in human neonates that is associated with high mortality and morbidity

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[9,10,16–18]. Disseminated disease with or without involvement of the central nervous system (CNS) and localized CNS disease account for the largest number of cases (75–88%) and the highest mortality rates (63–74%) in untreated cases. Intranasal inoculation of suckling or weanling mice with HSV type 2 (HSV-2) results in an infection that has many similarities to the disseminated and CNS disease in human neonates [5–7]. Initial replication of virus occurs in the respiratory tract with subsequent dissemination through the blood to visceral tissues, and spread to the brain by both viremia and neural transmission. This model infection has been very useful to determine efficacy of antiviral agents that have potential for use in human disease [5–7]. Idoxuridine was not effective in altering mortality or titers of virus in the CNS in the murine model [5], and this drug failed to alter mortality from herpes encephalitis in patients [1]. In contrast, both adenine arabinoside (ara-A) and adenine arabinoside 5′ monophosphate (ara-AMP) were shown to be effective in delaying viral replication and death in the murine model [6,7], and ara-A treatment reduced mortality significantly in HSV-infected neonates with CNS involvement [17,18]. Results of antiviral chemotherapy in the murine model of neonatal herpes, therefore, appear to be predictive of efficacy in the human disease.

Acyclovir (ACV) is a newly licensed antiviral agent which has been effective against HSV-1 and HSV-2 infections of the CNS in mice [4,8,11,13] and in treating HSV-1 encephalitis and genital HSV-2 infections in humans [2,12,15]. Clinical trials with ACV are currently in progress in patients with neonatal herpes. The purpose of this communication is to report the effect of parenteral (i.p.) or oral ACV therapy on mortality and viral titers in tissues in the murine model of neonatal herpes.

Materials and Methods

Animals and virus inoculation

3-week-old female Swiss Webster mice obtained from Simonsen Laboratories, Gilroy, CA, were inoculated by the intranasal route with the MS strain of HSV-2 by allowing each mouse to inhale 0.03 ml of virus dropwise from a 26-gauge needle as described previously [6,7]. Each animal received about 1.0×10^5 pfu resulting in an 80–90% mortality. To determine final mortality rates, animals were observed for 21 days after viral inoculation.

Virus, cell cultures, media, and virus assay

The virus pool used in these studies was prepared in secondary rabbit kidney (RK) cells and titered 2.0×10^7 pfu/ml when assayed in RK cells. The preparation of cell cultures, media utilized and assays for virus have been described previously [5].

Antiviral drug

The disodium salt of ACV utilized in this study was provided through the Antiviral Substances Program, NIAID, NIH, by Burroughs Wellcome Co., Research Triangle Park, NC. For i.p. administration mice were given 0.1 ml (60 mg/kg) twice daily for 7 days. For oral administration ACV was dissolved in the drinking water at a concentra-

tion of 1.5 mg/ml. Since mice in these experiments consumed 4–5 ml of water per day, each mouse received about 400 mg of ACV per kg per day for 7 days. As a general rule in our animal model experiments, we use the highest dose of drug that does not result in clinically evident toxicity. No toxicity was observed with the drug concentrations used in these experiments. Because the metabolism of ACV differs in different animal species [3], the doses used in the murine model cannot be compared with the dose used in humans.

Pathogenesis of infection

Three mice from an untreated control group and three from the ACV-treated group were killed daily through day 7 and on day 10. Blood was collected by cardiac puncture, and thoracic and abdominal organs were removed. The brain was removed and separated into olfactory lobe, cerebrum, and pons-medulla (pons). Individual tissues of the three mice from each group were pooled, and 10% homogenates were prepared on a wt./vol. basis in minimal essential medium containing 10% fetal bovine serum. The homogenates were centrifuged at 2500 rpm for 10 min; the supernatants were collected and stored at −70°C until assayed on RK cells for HSV.

Statistical evaluation

To compare the final mortality of placebo and ACV-treated mice, the data were evaluated using the Fisher exact test. The differences in the mean day of death (MDD) between placebo and ACV-treated animals were compared using the Mann-Whitney U-test. A P value of 0.05 or less was considered to be significant.

Results

Effect of treatment with ACV on mortality

After intranasal inoculation, animals developed ruffled fur and hunching on day 3–4, followed by paralysis and death between days 5 and 10. The results of treatment with ACV administered i.p. or orally on final mortality rates are shown in Table 1. In experiment 1 the control mice that received PBS by the i.p. route had a final mortality of 87% and a MDD of 7.5 days. When 60 mg of ACV per kg was administered i.p. twice daily for 7 days, protection was observed in groups of mice that received treatment beginning as late as 2 days after infection. In the second experiment, the control group had a final mortality of 90% and a MDD of 6.8 days. When ACV was added to the animal's drinking water, treatment was effective in protecting mice even when initiation of therapy was delayed until 4 days after HSV-2 inoculation. Complete protection was observed in the groups that received treatment beginning 1 or 2 days after infection.

Effect of treatment with ACV on viral pathogenesis

To define the mechanism of successful ACV therapy in this model infection, the titers of HSV-2 in tissues of both untreated and ACV-treated mice were determined (Fig. 1). In untreated control mice, virus was first detected in the lung on day 1 with

TABLE 1

Effect of treatment with acyclovir (ACV) on mortality of weanling mice inoculated intranasally with HSV type 2

Treatment	Mortality				
	No.	%	*P* value	MDD	*P* value
Expt. 1: i.p. treatment[a]					
PBS control	13/15	87	-	7.5	-
ACV + 1 h	4/15	27	<0.01	13.8	<0.01
+ 1 day	4/14	29	<0.01	12.8	<0.01
+ 2 d	2/15	13	<0.001	13.0	<0.05
+ 4 d	9/15	60	N.S.	7.1	N.S.
Expt. 2: oral treatment[b]					
H_2O control	27/30	90	-	6.8	-
ACV + 1 day	0/15	-	<0.001	-	-
+ 2 d	0/15	-	<0.001	-	-
+ 3 d	2/15	13	<0.001	8.0	<0.05
+ 4 d	6/15	40	<0.01	7.7	N.S.
+ 5 d	12/15	80	N.S.	7.1	N.S.

[a] 60 mg of ACV per kg i.p. twice daily for 7 days.

[b] ACV dissolved in drinking water at a concentration of 1.5 mg/ml. Each mouse received about 400 mg of drug per kg per day for 7 days.

subsequent spread to the spleen and kidney by day 6. During this period of time, virus also spread from the nasopharynx to the pons by day 3 and the olfactory lobe and cerebrum by day 5. In mice that received ACV in their drinking water beginning 1 day after infection, there was complete inhibition of HSV replication in the kidney, spleen, olfactory lobe, and cerebrum and in the lung after day 4. There was a 2–3 log decrease in titers of virus in the pons after day 4. Similar results were obtained in animals treated i.p. with ACV beginning 1 day after viral challenge (Fig. 2).

Discussion

The current report indicates that ACV therapy significantly reduced mortality, prolonged the MDD, and decreased replication of virus in tissues in the murine model of neonatal HSV-2 infection. Continuous oral therapy with drug in the drinking water was more effective than twice daily i.p. treatment in that oral drug significantly reduced mortality when begun as late as 4 days after viral challenge, while i.p. ACV was effective only as late as day 2. As reported previously [8], the increased effectiveness of oral therapy may be due to: (1) the higher total daily dose given orally (400 mg/kg vs. 120 mg/kg), and/or (2) the continuous (drug in drinking water) as opposed to intermittent (i.p. drug twice daily) administration of ACV which resulted in maintenance of effective levels of ACV in plasma and brain tissue.

It is of interest that ACV therapy was effective when begun as late as 4 days after

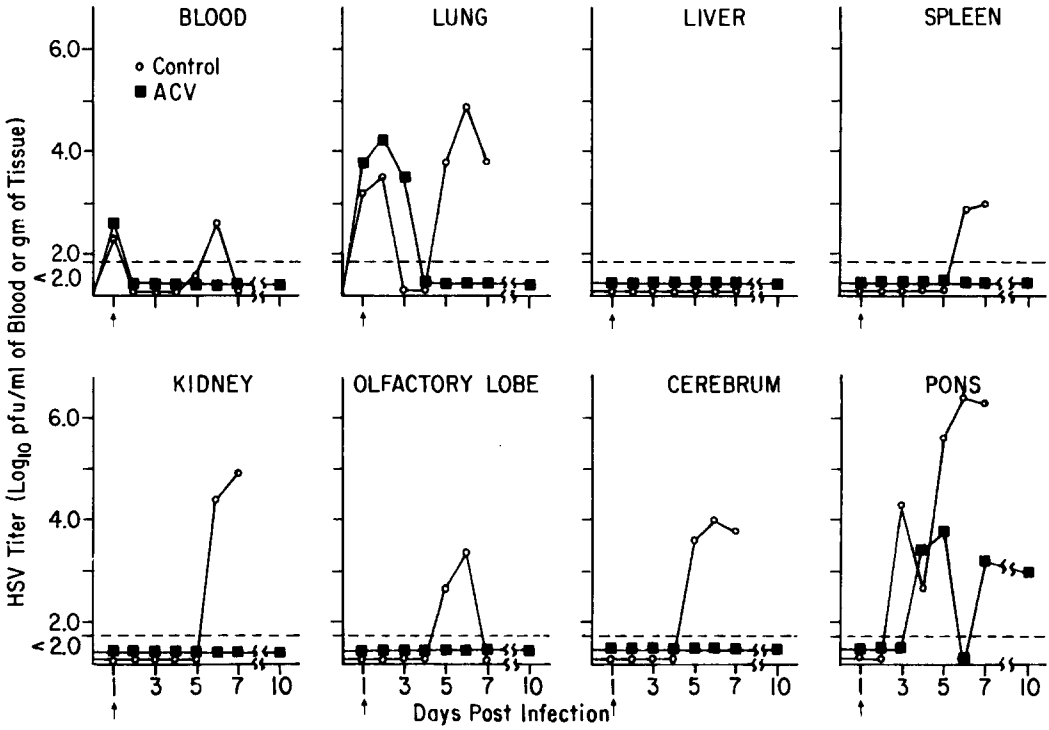

Fig. 1. Effect of oral treatment with acyclovir (ACV) on the viral pathogenesis of HSV-2 infection in weanling mice inoculated intranasally. Treatment with 400 mg of ACV per kg in drinking water for 7 days was initiated 1 day after viral challenge. Each point represents pooled tissues from 3 mice.

viral challenge when the current and previous studies from our laboratory [7] indicate that viral replication begins in CNS tissues by day 3 to 4. This suggests that ACV therapy may successfully treat HSV disease of the CNS even after viral replication is already well-established. Field and co-workers [4] also demonstrated that ACV successfully reduced mortality in a murine model of HSV-1 encephalitis when therapy was initiated after viral replication was already established in the brain. In the current report and in neonatal rabbits inoculated subcutaneously with HSV-2 [14], successful therapy with ACV was associated with inhibition of viral replication in several tissues. These combined results, therefore, suggest that there is a strong correlation between the ability of a drug to reduce mortality and the capacity to inhibit established replication of virus in tissues, particularly the CNS.

In our experiments, ACV appeared to be more effective in murine neonatal herpes than results reported previously [6,7] with adenine arabinoside (ara-A) or adenine arabinoside 5'-monophosphate (ara-AMP). Ara-A and ara-AMP delayed but did not reduce final mortality, and delayed but did not suppress viral replication in lung, liver, spleen, and brain. These results suggest that ACV may be more effective than ara-A or ara-AMP in neonatal HSV infections. It is of interest that recent studies by Skoldenberg and colleagues [15] and Whitley et al. [19] have indicated that ACV was signifi-

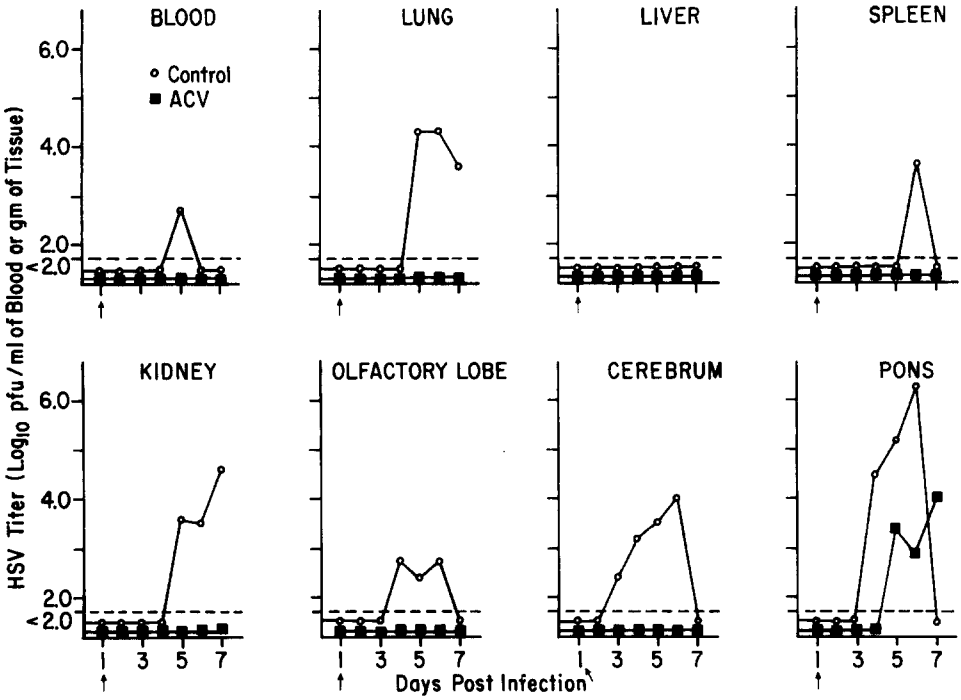

Fig. 2. Effect of i.p. treatment with acyclovir (ACV) on the viral pathogenesis of HSV-2 infection in weanling mice inoculated intranasally. Treatment with 60 mg of ACV per kg twice daily for 7 days was initiated 1 day after viral challenge. Each point represents pooled tissues from 3 mice.

cantly better than ara-A in the treatment of HSV encephalitis in older children and adults. While ara-A has been shown to significantly reduce mortality and decrease neurologic sequelae in neonatal herpes [17,18], there are too few published neonatal cases treated with ACV to fully assess efficacy with this drug [10]. The Collaborative Antiviral Study Group comparison of ara-A and ACV in neonates is nearing completion, and, until the results of this study are analyzed, we will not know whether the superior results with ACV over ara-A in the murine model is predictive of outcome in disseminated HSV-2 disease in humans.

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