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Alteration of Mortality and Pathogenesis of Three Experimental *Herpesvirus hominis* Infections of Mice with Adenine Arabinoside 5'-Monophosphate, Adenine Arabinoside, and Phosphonoacetic Acid†

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The therapeutic effectiveness of adenine arabinoside 5'-monophosphate (ara-AMP), adenine arabinoside (ara-A), and phosphonoacetic acid (PAA) was compared in three experimental *Herpesvirus hominis* type 2 infections of mice. In animals inoculated with *H. hominis* by the intracerebral or intraperitoneal route, both ara-AMP and ara-A were highly effective in reducing mortality even when treatment was begun 48 to 96 h after viral inoculation. ara-AMP was the most effective in both models in that treatment could be initiated 24 to 48 h later in the course of infection than with ara-A and still confer significant protection. In mice inoculated intraperitoneally, protection due to ara-AMP therapy was associated with reduced replication of virus in visceral organs and complete inhibition of transmission of virus to the brain. PAA treatment of mice inoculated intraperitoneally was effective in reducing mortality only if initiated shortly after infection. Treatment with PAA did not reduce mortality of mice inoculated intracerebrally but did prolong the mean day of death. When mice were inoculated intranasally with *H. hominis*, none of the three drugs altered final mortality; however, treatment with ara-AMP did prolong the mean day of death. Treatment with ara-AMP effectively reduced viral replication in the lung and liver in this model infection, but failed to prevent transmission of virus through the trigeminal nerves from the nasopharynx to the brain.

Herpesvirus hominis is the causative agent of a variety of diseases in humans, including localized vesicular and ulcerative lesions of the perioral or genital regions, and more severe, often fatal infections, including disseminated herpes in neonates or acute encephalitis in adults (22, 23, 27). Although a number of antiviral compounds have been tested both topically and systemically for therapeutic effectiveness in humans, parenteral administration of adenine arabinoside (ara-A) appears to be the most promising (3, 26, 33, 34). One of the major problems associated with systemic administration of ara-A is its water insolubility, which has necessitated the use of large volumes of fluid for its delivery. A phosphorylated derivative of ara-A, adenine arabinoside 5'-monophosphate (ara-AMP) is water soluble and has equal, or greater, efficacy as ara-A against *H. hominis* infections of tissue culture cells and in experimental animals (18, 29, 30, 32). Both drugs have been active when given systemically; however, with the exception of infections of the eye (26), they have not been effective when applied topically (1, 12, 18). In contrast, phosphonoacetic acid (PAA) is a compound that is active against herpesvirus in tissue culture (18, 25) and effective when applied topically to treat cutaneous or mucous membrane *H. hominis* infections of animals (8, 9, 18, 20 28). It has been only marginally active, however, when administered systemically to animals with herpesvirus infections (7, 8, 19). Because of its potential toxicity, PAA has not been tested in humans.

We reported previously that in a model genital *H. hominis* type 2 infection of mice, topical (intravaginal) treatment with PAA, but not ara-A or ara-AMP, was effective in reducing virus titers in vaginal secretions and in preventing mortality from ascending encephalomyelitis (18). None of the drugs was active when administered systemically. To further define the appropriate use of these three drugs

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in the treatment of *H. hominis* infections, we compared their effectiveness in three experimental *H. hominis* type 2 infections of the central nervous system (CNS) of mice in which the virus was inoculated by the intracerebral (i.c.), intraperitoneal (i.p.), or intranasal route. Previous studies in our laboratory (15–17) have demonstrated the importance of defining the pathogenesis of experimental *H. hominis* infections to determine the reasons for success or failure of a particular antiviral compound. The purpose of the current study was to determine: (i) the antiviral activity of systemically administered ara-AMP, ara-A, and PAA in all three model infections, and (ii) the alteration of the pathogeneis of each infection by treatment with ara-AMP.

MATERIALS AND METHODS

Animals and virus inoculation. Two-week-old Swiss Webster mice (Simonsen Laboratories, Gilroy, Calif.) were inoculated i.c. by injecting 0.03 ml of *H. hominis* type 2 into the right cerebral hemisphere. Each animal received approximately 2 to 5 plaque-forming units (PFU) of virus resulting in a 95 to 100% mortality in untreated control animals. Three-week-old female mice were inoculated by either the intranasal or i.p. route wih *H. hominis* type 2. Mice were inoculated i.p. with 2×10^4 PFU, which resulted in a 95 to 100% mortality. Intranasal inoculation was accomplished by allowing the mice to inhale 0.03 ml of virus from a 26-gauge needle, as described previously (17). Each animal received approximately 6.0×10^4 PFU, resulting in a 70 to 90% mortality. In all experiments the animals were observed for 3 weeks after viral inoculation.

Virus, media, cell cultures, and virus assay. The MS strain of *H. hominis* type 2 was obtained from Andre Nahmias, Emory University, Atlanta, Ga. The virus pool used in these studies was prepared in secondary rabbit kidney cells and titered 2.0×10^7 PFU/ml when assayed in rabbit kidney cells or fetal lamb kidney cells. The media utilized, preparation of cell cultures and assays for virus have been described previously (17).

Antiviral agents. The three compounds utilized in this study were obtained through the Antiviral Substances Program (National Institute of Allergy and Infectious Diseases, Bethesda, Md.). The disodium salt of PAA was provided by Abbott Laboratories, North Chicago, Ill. ara-A and ara-AMP were furnished by Parke-Davis and Co., Ann Arbor, Mich. Both PAA and ara-AMP were dissolved in phosphate-buffered saline (PBS), and ara-A was suspended in 0.4% carboxymethyl cellulose. All drugs were prepared just before use and were administered i.p. in a volume of 0.1 ml. The maximum tolerated dose given twice daily for 7 days without obvious toxicity was 250 mg/kg for ara-A and ara-AMP and 250 to 500 mg/kg for PAA. Therefore, a dose of 250 mg/kg was used for all three drugs in most experiments. In some experiments where this regimen was not effective a higher dose was used.

Pathogenesis of the infections. Three mice from untreated control groups and three from treated groups were sacrificed daily. Organs were harvested and pooled by tissue type from each group on days 1 through 6 in mice inoculated i.c., on days 2 through 10 in mice infected i.p., and on days 1 through 8 in mice infected intranasally. Blood was collected by cardiac puncture, and thoracic and abdominal organs were removed. The sample referred to as gut includes the colon and cecum only. Olfactory lobe, cerebrum, and cerebellum-brain stem including upper cervical spinal cord were dissected out, and the balance of the spinal cord was removed. In mice inoculated i.c., the brain was separated into olfactory lobe, cerebral cortex, diencephalon, cerebellum, and pons-medulla. Ten percent homogenates of the above tissues were prepared on a weight/volume basis in minimal essential medium containing 10% fetal bovine serum. The homogenates were centrifuged at 2,500 rpm for 10 min; supernatants were removed and stored at −70°C until assayed on fetal lamb kidney cells for the presence of virus.

Statistical evaluation. To compare the final mortality of control and drug-treated mice, the data were evaluated by the Fisher Exact test. To evaluate the differences in mean day of death (MDD) between control and drug-treated animals, the data were compared by the Mann-Whitney U test. A P value of <0.05 was considered to be significant.

RESULTS

Effect of treatment with ara-AMP, ara-A, or PAA on mortality of mice inoculated i.c. with *H. hominis* type 2. After i.c. inoculation of approximately 2 PFU of virus, mice became paralyzed on days 5 to 7 and died on days 6 to 8. The effect of treatment with 250 mg of ara-AMP, ara-A, or PAA per kg given i.p. twice daily for 7 days is summarized in Table 1. The virus control group treated with PBS had a final mortality of 97% and a MDD of 7.5 days. Treatment with ara-AMP was highly effective in reducing final mortality and increasing the MDD when administered as late as 72 h after infection. ara-A was also effective in reducing final mortality when administered as late as 48 h after virus inoculation. Therapy with PAA was not effective in reducing final mortality but did prolong the MDD at all the treatment times.

Effect of treatment with ara-AMP on pathogenesis of *H. hominis* infection of mice inoculated i.c. To define the mechanism of successful drug therapy in this experimental infection, the pathogenesis of infection in both untreated and ara-AMP-treated mice was determined (Fig. 1). In the untreated control animals, virus was first detected in the cerebral cortex, diencephalon, cerebellum, and pons and medulla on day 2. Virus titers in these organs

TABLE 1. *Effect of treatment with ara-AMP, ara-A, or PAA on mortality of 2-week-old mice inoculated i.c. with H. hominis type 2*

Treatment[a]	Mortality		MDD ± SD
	No.	%	
PBS	36/37	97	7.5 ± 1.6
ara-AMP			
+1 h	14/34	41[b]	9 9 ± 2.4[b]
+24 h	14/34	41[b]	9.9 ± 1.8[b]
+48 h	10/26	38[b]	9.8 ± 2.0[b]
+72 h	6/16	38[b]	9.8 ± 2.0[c]
Drug control	0/20		
ara-A			
+1 h	15/34	44[b]	11.5 ± 2.3[b]
+24 h	9/24	26[b]	11.6 ± 4.2[b]
+48 h	13/26	50[b]	9.1 ± 3.1
+72 h	14/16	88	8.5 ± 2.7
Drug control	0/20		
PAA			
+1 h	31/34	91	9.2 ± 2.1[b]
+24 h	33/34	97	8.6 ± 2.2[d]
+48 h	10/10	100	8.4 ± 1.2[d]
Drug control	0/20		

[a] 250 mg of drug per kg i.p. twice daily for 7 days.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] $P < 0.05$.

ranged from 10^3 to 10^4 PFU/g and gradually increased until the death of the mice. Virus was detected in the olfactory lobe and in lung on day 4. In the animals in which ara-AMP treatment was initiated 24 h after inoculation, there was reduction in viral replication in all the CNS tissues examined. On day 5 when most of the control animals were dying only low levels of virus were present in the CNS of ara-AMP-treated mice. Survival of the treated animals appeared to be due to inhibition of viral replication within the CNS.

Effect of treatment with ara-AMP, ara-A, or PAA on the mortality of mice inoculated i.p. with *H. hominis* type 2. After i.p. inoculation of 10^4 PFU of virus, mice developed ruffled fur and hunching on days 5 to 6. Some animals then developed hind leg paralysis, and most of the mice died between 7 and 10 days after inoculation. The results of treatment in this model infection with 500 mg of ara-AMP per kg i.p. once daily for 5 days or 250 mg of ara-A or PAA per kg i.p. twice daily for 7 days are summarized in Table 2. Treatment with ara-AMP was highly effective in reducing final mortality when initiated as late as 96 h after infection. ara-A also reduced mortality when given up to 48 h after infection and prolonged the MDD when administered as late as 72 h after virus inoculation. PAA treatment decreased mortality only when given within 3 h after infection.

Effect of ara-AMP on pathogenesis of *H. hominis* infection of mice inoculated i.p. Since ara-AMP was also the most effective of the three drugs in this model, the effect of ara-AMP treatment on pathogenesis was investigated to define the mechanisms of successful therapy. The pathogenesis of this infection in both untreated and ara-AMP-treated mice is illustrated in Fig. 2. In untreated infected animals, virus was first detected in gut, liver, and spleen on day 2, in lung on day 5, in kidney, cerebellum-brain stem, and cerebrum on day 6, and in the spinal cord on day 7. Virus was isolated from the blood only on days 7 and 9. In mice treated with ara-AMP beginning 24 h after viral inoculation, initial replication of virus in gut and spleen was markedly inhibited, replication in lung and liver was com-

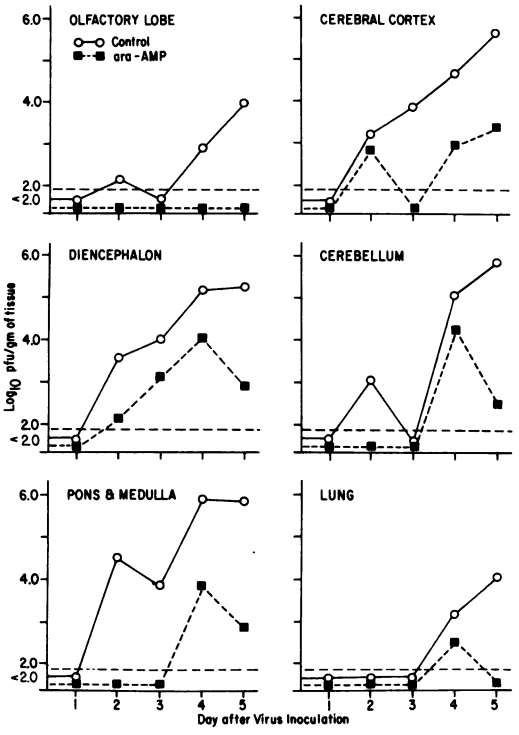

FIG. 1. *Effect of treatment with ara-AMP on pathogenesis of H. hominis type 2 infection in 2-week-old mice inoculated i.c. Treatment with a 250-mg/kg dose i.p. twice daily for 7 days was initiated 24 h after viral inoculation. Symbols: (○) control; (■) treated with ara-AMP.*

TABLE 2. *Effect of treatment with ara-AMP, ara-A, or PAA on mortality of 3-week-old mice inoculated i.p. with H. hominis type 2*

Treatment	Mortality		MDD ± SD
	No.	%	
Expt 1			
PBS	35/36	97	9.2 ± 3.2
ara-AMP[a]			
+3 h	13/35	37[b]	15.2 ± 4.4[b]
+24 h	12/34	35[b]	10.8 ± 4.0[c]
+48 h	12/35	34[b]	13.2 ± 5.3[d]
+72 h	6/20	30[b]	10.3 ± 4.8
+96 h	11/20	55[b]	10.3 ± 3.1
Drug control	0/15		
Expt 2			
PBS	15/15	100	8.6 ± 1.1
ara-A[e]			
+3 h	2/15	13[b]	10.5 ± 3.5
+24 h	7/15	47[d]	14.3 ± 4.0[b]
+48 h	8/14	57[c]	13.0 ± 3.4[b]
+72 h	10/14	71	11.7 ± 3.9[c]
Drug control	0/10		
PAA[e]			
+3 h	3/12	25[b]	10.3 ± 2.5
+24 h	11/14	79	9.2 ± 1.7
+48 h	14/14	100	9.7 ± 2.6
Drug control	0/15		

[a] 500 mg of drug per kg i.p. once daily for 5 days.
[b] $P < 0.001$.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] 250 mg of drug per kg i.p. twice daily for 7 days.

pletely inhibited, and there was no transmission of virus to the CNS. The ability of ara-AMP treatment to protect mice against mortality in this infection appeared to be due to suppression of viral replication in the initial target organs and prevention of spread from these organs to the CNS.

Effect of ara-AMP, ara-A, or PAA treatment on mortality of mice inoculated intranasally with *H. hominis* type 2. After intranasal inoculation of 3-week-old mice with 5×10^4 PFU, the animals developed ruffled fur and hunching on days 3 to 4, followed by paralysis and death between days 5 and 10. The results of treatment with 500 mg i.p. of ara-AMP or ara-A per kg twice daily for 7 days or 250 mg i.p. of PAA per kg twice daily for 7 days are listed in Table 3. In the first experiment, virus-infected mice treated with PBS had a final mortality of 70% and a MDD of 8.9 days. Treatment with ara-AMP did not decrease final mortality but did prolong the MDD. In the second experiment, infected mice treated with PBS had a final mortality of 87% and a MDD of 6.1 days. Neither ara-A per kg nor PAA treatment was effective in reducing mortality or prolonging the MDD.

Effect of ara-AMP treatment on pathogenesis of *H. hominis* infection of mice inoculated intranasally. Since ara-AMP treatment was highly effective against *H. hominis* type 2 infection initiated by the i.c. or i.p. routes but failed to alter the infection initiated intranasally, studies on the pathogenesis of the infection were performed to determine the reasons for the failure of ara-AMP. The pathogenesis of this infection in both control and ara-AMP-treated animals is illustrated in Fig. 3. In the control animals, virus was first detected in the lung on day 2 with subsequent spread, probably through blood, to liver and spleen. During this period of time virus also spread from nasopharynx to olfactory lobe, cerebellum-brain stem, cerebrum, and spinal cord. In another series of experiments, virus was detected in the trigeminal nerve 24 h after inoculation of virus and 48 h before its detection in the pons-medulla or other parts of the CNS. In mice inoculated with *H. hominis* and treated with ara-AMP 1 h later, marked inhibition of viral replication was observed in lung and liver. In most other tissues, including the trigeminal nerves and those of the CNS, a 1- to 2-day delay in viral replication occurred, correlating with the 1- to

TABLE 3. *Effect of treatment with ara-AMP, ara-A, or PAA on mortality of 3-week-old mice inoculated intranasally with H. hominis type 2*

Treatment	Mortality		MDD ± SD
	No.	%	
Expt 1			
PBS	21/30	70	8.9 ± 2.2
ara-AMP[a]			
+1 h	16/29	55	9.8 ± 3.1
+24 h	18/29	62	12.3 ± 4.1[b]
+48 h	17/30	57	10.4 ± 2.4[c]
Drug control	0/20		
Expt 2			
PBS	13/15	87	6.1 ± 2.1
ara-A[a]			
+1 h	11/12	92	6.6 ± 2.3
+24 h	14/14	100	7.3 ± 2.6
Drug control	0/15		
PAA[d]			
+1 h	12/12	100	6.5 ± 2.6
+24 h	9/9	100	7.1 ± 1.9
Drug control	0/15	13	

[a] 500 mg of drug per kg i.p. twice daily for 7 days.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] 250 mg of drug per kg i.p. twice daily for 7 days.

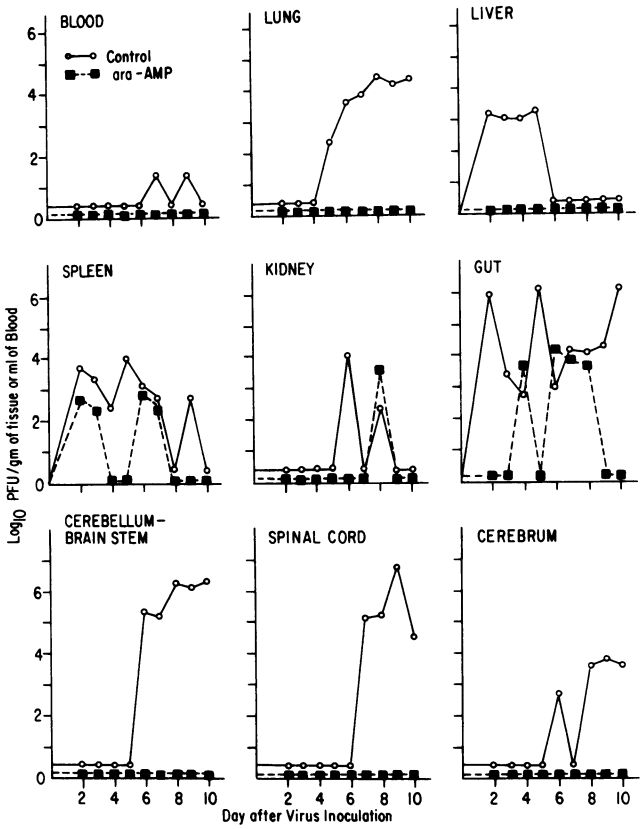

FIG. 2. *Effect of treatment with ara-AMP on pathogenesis of H. hominis type 2 infection in 3-week-old mice inoculated i.p. Treatment with a 500-mg/kg dose i.p. once daily for 5 days was initiated 24 h after viral inoculation. Symbols:* (○) *control;* (■) *treated with ara-AMP.*

2-day delay in the MDD of ara-AMP-treated animals.

DISCUSSION

We previously compared the sensitivity of *H. hominis* types 1 and 2 to ara-AMP, ara-A, and PAA in tissue culture cells (18). Type 2 strains were two- to four-fold more sensitive to ara-AMP and ara-A than to PAA; however, PAA was clearly the most effective drug in the topical treatment of genital *H. hominis* type 2 infections of mice. In the present study ara-AMP and ara-A were considerably more effective than PAA in the systemic treatment of *H. hominis* type 2 infections initiated by i.c. or i.p. routes. These data indicate that the relative activity of a compound in vitro may not always be predictive of effectiveness in vivo and that therapeutic efficacy may be influenced by route of inoculation of the virus and pathogenesis of the infection. Other factors such as drug solubility, tissue penetration and distribution, and rate of degradation and excretion also play a role in the success or failure of therapy in vivo (10).

In mice inoculated by the i.c. route, treatment with either ara-AMP or ara-A was very effective. ara-AMP appeared to be more effective than ara-A in that treatment could be delayed until later in the course of infection and still result in significant protection. These results agree with those reported by others (29, 30). Successful therapy with ara-AMP in the present study was associated with a significant reduction in the titers of virus in all tissues of the CNS. Virus titers in the CNS of treated animals continued to decline through day 5 and presumably would have been eliminated had the experiment been extended for additional days. A similar reduction of virus in the brains of mice inoculated i.c. with *H. hominis* type 1 and treated subcutaneously with ara-A has been reported by Griffith and associates (13). In our experiments treatment with PAA was ineffective in reducing mortality but did prolong the MDD. Other studies with this com-

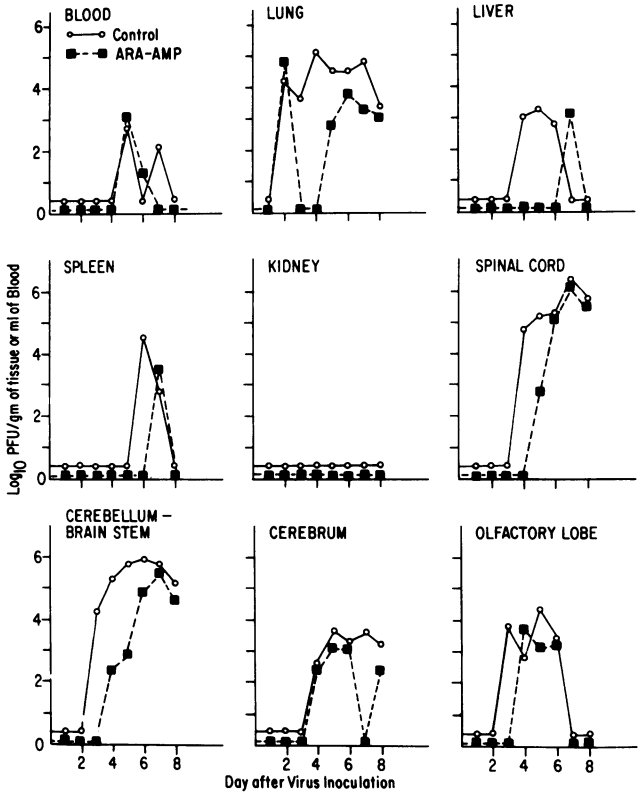

FIG. 3. *Effect of treatment with ara-AMP on pathogenesis of H. hominis type 2 infection in 3-week-old mice inoculated intranasally. Treatment with a 500-mg/kg dose i.p. twice daily for 7 days was initiated 1 h after viral inoculation. Symbols:* (○) *control;* (■) *treated with ara-AMP.*

pound have also shown only marginal activity against *H. hominis* type 1 inoculated by the i.c. route (7).

When mice were infected by the i.p. route, ara-AMP was also more effective than ara-A; ara-AMP prevented mortality when given up to 96 h and ara-A only as late as 48 h after infection. In contrast, PAA was effective only when given shortly after inoculation of virus. It should be noted that although this model does not simulate a natural route of infection for humans, it has proven to be the most sensitive of the experimental infections we have utilized for evaluation of antiviral agents. Additionally, it offers a convenient model for the preliminary screening of potential anti-herpesvirus compounds. The pathogenesis of the infection is characterized by initial viral replication in gut, liver, and spleen after direct invasion of the virus from the peritoneal cavity. Although we postulate that secondary spread to lung, kidney, and CNS is primarily by the hematogenous route, we were unable to isolate virus from blood before day 7 in the experiments reported here. In other experiments, however, virus was isolated from blood in low titers as early as day 4. It should be noted that very low levels of virus cannot be detected since blood samples had to be diluted to prevent toxicity to tissue culture cells. Further support for a viremic phase in this model comes from our experiments which demonstrated that silica altered the susceptibility of mice to i.p. inoculation of *H. hominis* (21). In these experiments the entire infection was enhanced, and virus was isolated from blood on day 2. The concept of viremic spread of *H. hominis* from peritoneal cavity to the CNS is further supported by the experiments of Johnson (22). He reported that the primary mechanism of transmission of *H. hominis* type 1 to the CNS of suckling mice infected i.p. with the virus is by hematogenous spread, but that direct neural transmission from visceral organs could also occur. In our mice infected by the i.p. route and treated with ara-AMP, there was a marked decrease in the initial viral replication in gut, liver, and spleen and complete inhibition of

spread to lung and to the CNS. Ara-AMP treatment, therefore, appeared to suppress initial viral replication to sufficiently low levels that a viremia and subsequent spread to the CNS did not occur. In groups of animals treated with ara-AMP or ara-A, we have consistently observed a 10 to 40% failure rate with an increased MDD of treated animals. It is possible that in these animals transmission of virus to the CNS was by direct neural spread and that treatment was not effective against transmission of virus by this route. Other possible explanations for failure include differences in drug disposition and metabolism in individual animals, variability in host resistance to infection, and differences in antibody or interferon levels.

We previously reported that in newborn mice inoculated intranasally with *H. hominis* type 2, treatment with ara-A, ara-C, iododeoxyuridine or polyinosinic-polycytidylic acid was not effective in reducing final mortality, although in some cases there was a significant alteration of the pathogenesis of infection, particularly in lung, liver, and spleen (15, 16, 17). We postulated that the reason for failure of drug therapy to protect the animals was the inability of treatment to prevent direct neural spread of the virus from nasopharynx to the CNS. In contrast, De Clercq and co-workers, using 10-day-old mice inoculated intranasally with *H. hominis* type 1, reported that these same drugs appeared to have some effectiveness (5). One possible explanation for the difference between these studies is that the type 2 strain we utilized has a greater capacity for neurovirulence than the type 1 strain used by De Clercq et al. The present studies appear to confirm our original hypothesis (15–17) that failure of therapy is due to inability of drug treatment to prevent direct neural spread to the CNS. In ara-AMP-treated animals, spread of virus through the trigeminal nerve was delayed but not completely inhibited, and similar delays in onset of viral replication were observed in the olfactory lobe, cerebellum-brain stem, and spinal cord. In contrast, significant inhibition of viral replication occurred in lung and liver tissue. These results are consistent with the 1- to 2-day delay in the MDD but lack of alteration of final mortality in ara-AMP-treated animals.

Both ara-AMP and ara-A were effective in the treatment of mice infected with *H. hominis* by the i.c. and i.p. but not intranasal or intravaginal (18) routes. It appears that the pathogenesis of the two former infections involves spread of virus to the CNS by the blood-borne or another route that is amenable to interruption by treatment with these two drugs. This hypothesis is supported by studies in other laboratories in which therapy of i.c. or i.p. herpes infections with passive antibody has reduced mortality (2, 4, 6, 11, 31). Therapy with ara-AMP and ara-A may be unable to alter *H. hominis* infections initiated by the intranasal or intravaginal route because virus is transmitted from infected mucous membranes directly to the CNS by peripheral nerves (current study; 18, 24), and the two drugs do not reach sufficient concentration in the peripheral nervous system to interrupt transmission or inhibit viral replication. If this hypothesis is true, one would not expect treatment with ara-AMP or ara-A to be effective in eradicating latent virus in neural ganglia.

Our data indicate that ara-AMP was more effective than ara-A in the treatment of the two *H. hominis* infections of mice where both compounds worked. This observation, plus the greater water solubility of ara-AMP, suggests that this compound may be of even greater therapeutic value than ara-A in the treatment of severe herpesvirus infections of humans.

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