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Herpesvirus hominis Infection in Newborn Mice: Comparison of the Therapeutic Efficacy of 1-β-D-Arabinofuranosylcytosine and 9-β-D-Arabinofuranosyladenine[1]

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Intranasal inoculation of newborn mice with *Herpesvirus hominis* type 2 provides an experimental infection that closely resembles disseminated herpesvirus infection of human newborn infants. After inoculation of mice, the virus multiplies in the respiratory tract and is disseminated through the blood to the liver and spleen and to the brain by both a viremia and nerve route transmission. Although therapy with 1-β-D-arabinofuranosylcytosine (ara-C) did not reduce final mortality, it did increase the mean survival time by 1 day. This effect on the mean survival time was associated with a 1-day delay in the appearance of herpesvirus in the blood, liver, and spleen and a reduction of virus replication in lung and brain for 1 day as compared with untreated control animals. Treatment with 9-β-D-arabinofuranosyladenine (ara-A) likewise had no effect on final mortality, but increased the mean survival time by 2 days. Therapy with ara-A delayed or suppressed virus replication in blood, lung, liver, spleen, and brain for 2 days. Although treatment with either ara-C or ara-A in this experimental *H. hominis* type 2 infection resulted in a temporary delay and/or suppression of viral replication in several target organs, neither compound was completely effective in inhibiting viral replication or in protecting animals from eventual death due to the infection.

Herpesvirus hominis (HVH) is the causative agent of several life-threatening diseases in humans including encephalitis in adults (22), and a generalized infection of neonates with or without central nervous system involvement (20, 23). Although infection of human newborn infants with HVH type 2 occurs relatively infrequently, it is associated with a 70% mortality rate and neurological sequelae in 50% of the survivors (20). In addition, HVH type 2 is a common cause of venereally transmitted genital infections in both males and females and has been associated with carcinoma of the cervix in females (21). There is clearly a need, therefore, for an antiviral chemotherapeutic agent in the treatment of human herpesvirus infections.

Although the most widely utilized form of therapy in newborn infants has been 5-iodo-2'-deoxyuridine (IUdR), the effectiveness of this compound has not been established clearly (20). Recently, two placebo-controlled, double-blind multicenter studies initiated to evaluate the effectiveness of IUdR in treatment of HVH encephalitis were terminated due to the severe toxicity associated with the use of this drug (L. T. Ch'ien, personal communication). In a previous detailed evaluation of IUdR in an experimental HVH type 2 infection of newborn mice, we reported that this drug was ineffective in reducing mortality because of an inability to control viral replication in the central nervous system (15). Workers from other laboratories have also demonstrated the failure of IUdR to inhibit herpesvirus replication in the central nervous system (25, 27).

In recent years, two drugs that inhibit the replication of deoxyribonucleic acid (DNA) viruses have been shown to be effective against HVH both in vitro and in vivo. 1-β-D-Arabinofuranosylcytosine (ara-C) has been reported to inhibit both HVH type 1 and type 2 in a variety of cell cultures (5, 10, 34) and to be as effective as IUdR in the treatment of experimental HVH keratitis (14, 33), and has been used in the treatment of HVH encephalitis in humans (8, 12). The purine nucleoside 9-β-D-arabinofuranosyladenine (ara-A) is active against HVH types 1 and 2 in cell cultures (18, 26) and has been reported to be effective against

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HVH keratitis in hamsters (30) and HVH encephalitis in hamsters and mice (1, 19, 29, 31, 32). Encouraging results have also been reported in the treatment of serious human HVH infections with ara-A (7).

The purpose of this study was to compare the therapeutic efficacy of ara-C and ara-A in an experimental infection of newborn mice with HVH type 2, which closely simulates disseminated HVH infections of human neonates (15). Experiments were designed to: (i) compare the sensitivity to ara-C and ara-A of the MS strain of HVH type 2 utilized in these studies with other strains of HVH, (ii) determine the maximum tolerated dosages of each drug in newborn mice, (iii) evaluate the therapeutic efficacy of each compound by utilizing various treatment schedules, and (iv) determine the effect of treatment on the pathogenesis of the infection.

MATERIALS AND METHODS

Animal model. Newborn mice (5 to 7 days of age) from pregnant Swiss Webster females (Simonsen Laboratories, Gilroy, Calif.) were inoculated intranasally by allowing each mouse to inhale six drops (approximately 0.01 ml) of HVH type 2 from a 26-gauge needle. Each animal received approximately 1,000 plaque-forming units (PFU) (8 mean lethal doses) of herpesvirus resulting in a 90 to 100% mortality. When lower mortality rates were desired, appropriate dilutions of the inoculum were utilized.

Viruses. The type 2 MS strain of HVH utilized in these studies was obtained from Andre Nahmias, Emory University, Atlanta, Ga. The virus pool used was propagated in primary rabbit kidney cells and titered 10^6 PFU/ml. Other type 2 strains, including Alabama, Curtis, Ellison, and Lovelace, and three type 1 strains, Shealey, Tyler, and VR_3, were also obtained from A. Nahmias. Viruses isolated in our own laboratory from genital lesions include Holt, Turner, and Jensen. Although these isolates have not been fully characterized, they are probably type 2.

Media, cell cultures, and virus assays. The media utilized and the preparation of cell cultures (primary mouse embryo fibroblasts, human foreskin fibroblasts, and fetal lamb kidney cells) have been described previously (15). Human embryonic lung (WI-38) cells were obtained from Leonard Hayflick, Stanford University, Palo Alto, Calif. Plaque titrations of HVH were performed as described previously (15).

Antiviral drugs. ara-C (Cytosar, Cytarabine) was kindly supplied by The Upjohn Company, Kalamazoo, Mich. The lyophilized powder was reconstituted with the diluent supplied, and diluted in phosphate-buffered saline to obtain the desired concentrations. The drug was administered in a volume of 0.05 ml by the intraperitoneal route. Micronized ara-A was prepared by Parke, Davis & Co., Detroit, Mich., and supplied through the Antiviral Substances Program of the National Institute of Allergy and Infectious Diseases, Bethesda, Md. The drug was stored as a powder at 4 C and prepared just prior to use. Since the drug is relatively insoluble, it was administered intraperitoneally, in appropriate concentrations, as a suspension in 0.4% carboxymethylcellulose (Sigma Chemical Co., St. Louis, Mo.) in a volume of 0.05 ml.

Assays for drug activity. The susceptibility of the HVH strains to ara-C and ara-A was determined by mixing serial twofold dilutions of the appropriate drug in twice-concentrated minimal essential media with an equal volume of 1.0% agarose. The overlay mixture containing drug was applied to monolayer cultures of primary mouse embryo fibroblasts, human foreskin fibroblasts, or WI-38 cells 1 h after infection with 50 to 100 PFU of each of the herpesvirus strains. After 48 h of incubation the cells were stained with neutral red and plaques were counted. The minimum inhibitory level was defined as the concentration of drug that resulted in a 50% reduction of the number of plaques in the control plates. The level of activity of the drugs in mouse serum or tissue was measured by preparing twofold dilutions of serum or 10% tissue homogenates in twice-concentrated minimal essential medium mixing with an equal volume of 1.0% agarose, and applying the overlay mixture to monolayers of mouse embryo fibroblasts cells infected with the MS strain of HVH. Serial dilutions of known drug concentrations were run with each assay. In addition, serum and tissue homogenates from control animals that did not receive drug were included to determine any nonspecific inhibition due to the tissue homogenates.

Statistical evaluation. To statistically evaluate the differences in the mean survival times between drug-treated and untreated animals, the data were compared by use of Student's t test. To compare the final mortalities of drug-treated and untreated mice, the data were evaluated by chi-square analysis. A P value of <0.05 was considered to be significant.

RESULTS

Susceptibility of HVH strains to ara-C and ara-A. The relative susceptibility of HVH strains to antiviral agents has been reported to be variable depending upon the antigenic type and passage history of the virus, the cell culture system utilized, and the method employed to determine susceptibility. To determine whether the MS strain utilized in our animal experiments was representative of other HVH strains, we compared its susceptibility to ara-C and ara-A with four other reference type 2 strains, three reference type 1 strains, and three recent clinical isolates from genital lesions. Susceptibility to both compounds was determined for each virus strain in both mouse (mouse embryo fibroblasts) and human (human foreskin fibroblasts, WI-38) cells by utilizing a plaque reduction assay. The mean values from two separate experiments are provided in Table 1. In all three cell lines, the MS strain was about as suscepti-

ble to ara-C or ara-A as the reference type 1 and type 2 strains and the genital isolates. There appeared to be no difference in susceptibility between the type 1 and the type 2 strains to either ara-C or to ara-A in any of the cell lines. All strains appeared to be somewhat more susceptible to ara-C in human than in mouse cells, but with ara-A the susceptibility was slightly greater in mouse than human tissue. Under the conditions employed in our assay system, the HVH strains tested were about 10-fold more susceptible to ara-C than to ara-A. However, higher concentrations of ara-A than ara-C could be employed without cell toxicity as determined by microscope observation and uptake of neutral red.

Pathogenesis of infection. Inoculation of 5- to 7-day-old mice with 1,000 PFU (8 mean lethal doses) of HVH type 2 by the intranasal route resulted in a generalized infection, followed by paralysis, and death 5 days after infection. The pathogenesis of the infection has been described in detail previously (15) and is characterized by primary virus replication in the respiratory tract. A viremia, probably initiated from the lung, is first detectable at 36 to 48 h; liver, spleen, and brain are then seeded with virus from the blood. Virus is also transmitted to the central nervous system by way of the olfactory and trigeminal nerves.

Treatment with ara-C. The effects of ara-C on the mortality of HVH-infected mice were evaluated using various dosages and treatment schedules. Treatment by the intraperitoneal route was initiated 1 to 2 h after infection and was continued for a period of 5 days, at which time most animals were showing signs of paralysis. The maximum concentration of ara-C that could be administered daily for 5 days without lethal toxicity for suckling mice was 50 mg/kg per day. The effectiveness of this compound was determined for concentrations of 25 mg/kg given twice daily (at 12-h intervals) or 12.5 mg/kg administered four times daily (at 6-h intervals) utilizing 40 animals in each treatment group. The results of treatment utilizing 25 mg/kg twice a day are illustrated in Fig. 1. The untreated virus control group had a final mortality of 81% with a mean survival time of 4.6 days. The group that received treatment with ara-C immediately after infection had a final mortality of 83% with a mean survival time of 5.5 days. Although ara-C was not effective in reducing final mortality, it did significantly increase the mean survival time ($P < 0.001$). Treatment with 12.5 mg/kg administered four times a day produced similar results.

Treatment with ara-A. To evaluate the therapeutic efficacy of ara-A in this model infection, various concentrations of the drug were administered either two or four times daily beginning immediately after infection and con-

TABLE 1. *Susceptibility of type 1 and type 2 reference strains and genital isolates of HVH to ara-C and ara-A in mouse embryo fibroblasts (MEF), human foreskin fibroblasts (HFF), and human embryonic lung (WI-38) cell cultures*

Strain of HVH	50% Inhibitory levels[a] (μg/ml)					
	ara-C			ara-A		
	MEF	HFF	WI-38	MEF	HFF	WI-38
HVH type 1						
Shealy	0.3	0.08	0.05	5	25	9
Tyler	0.4	0.08	<0.05	5	>25[b]	5
VR_3	0.4	0.15	0.3	12	>25	>25
HVH type 2						
Alabama	0.8	0.15	0.3	5	18	18
Curtis	0.3	0.08	0.15	9	18	18
Ellison	0.6	0.15	0.3	5	18	12
Lovelace	0.4	0.15	0.15	5	>25	18
MS	0.6	0.3	0.15	9	>25	18
Genital isolates						
Holt	0.3	0.2	0.15	9	18	18
Jensen	0.3	0.2	0.15	5	25	18
Turner	0.4	0.15	0.15	9	>25	18

[a] Concentration of drug that reduced virus control plaque count by 50%.
[b] Highest concentration tested.

tinued for 5 days. The highest concentration of ara-A that could be administered for the desired length of time without being toxic was 1,000 mg/kg per day. The therapeutic effectiveness for this compound was determined, therefore, for treatment regimens of 500, 250, and 125 mg/kg given twice daily or 250, 125, and 62.5 mg/kg administered four times daily utilizing 40 animals in each treatment group. The results from the most effective treatment regimen, 500 mg/kg twice daily for 5 days, are presented in Fig. 2. The untreated virus control group had a final mortality of 87% with a mean survival time of 5.5 days. Animals that received ara-A had a final mortality of 83% with a mean survival time of 7.5 days. Treatment with ara-A did not effectively reduce final mortality; however, it did significantly increase mean survival time ($P < 0.001$). None of the other treatment regimens was more effective in either altering final mortality or increasing mean survival time. Treatment for longer than 5 days increased drug toxicity without increasing efficacy.

Effect of ara-C treatment on pathogenesis. We have reported previously (15) that, although

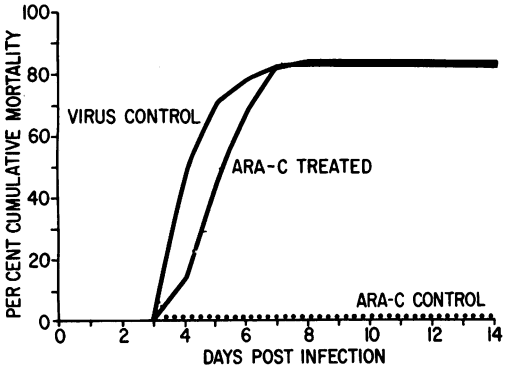

FIG. 1. *Effect of ara-C (25 mg/kg twice daily) on the mortality of HVH-2 infection of newborn mice.*

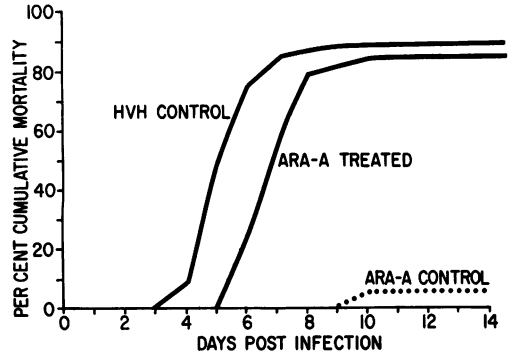

FIG. 2. *Effect of ara-A (500 mg/kg twice daily) on the mortality of HVH-2 infection of newborn mice.*

the HVH strain utilized in this experimental infection was highly susceptible to IUdR in tissue culture, treatment with this drug failed to decrease the mortality of newborn mice infected intranasally with this virus. Therapy with IUdR did, however, appreciably alter the pathogenesis of the infection. To determine why ara-C was not effective in reducing mortality in HVH-infected mice, similar studies of the effect of treatment on the pathogenesis of the infection were performed. Animals were inoculated with HVH type 2 and given twice daily injections of 25 mg of ara-C intraperitoneally per kg beginning immediately after virus inoculation. At 24-h intervals after virus challenge, 10 animals from a treated group and 10 animals from an untreated group were sacrificed. The animals were bled, organs were removed and homogenized, and the samples were assayed for virus. In the control samples (Fig. 3A), virus was first detected in the lung, spleen, liver, and blood by 48 h, but not in the brain until 72 h. Virus titers reached levels of 10^6 PFU/g of tissue in the lung, 10^4 to 10^5 PFU/g in the liver and spleen, 10^2 PFU/ml in the blood, and 10^5 to 10^6 PFU/g in the brain during the course of the infection. In the ara-C-treated animals there was a moderate alteration of pathogenesis (Fig. 3B). Virus replication in the lung was suppressed at 48 h when compared to the control (from 10^6 to 10^4 PFU/g of tissue). There was delay in the initial appearance of virus in the liver and spleen from 48 to 72 h and in blood from 48 to 96 h, but once replication occurred it appeared to be the same level as in the control animals. Since virus replication was observed in the liver and spleen of treated animals prior to its demonstration in the blood, it seems likely that there were subdetectable levels of virus (<10 PFU/ml) in the blood at the earlier time periods. Although there was no delay in the appearance of virus in the brain, there was a slight reduction in the amount of virus present at both 72 and 96 h (from 10^5 to 10^6 PFU/g in control animals to 10^4 to 10^5 PFU/g in treated animals). The effects of ara-C on the pathogenesis of HVH infection in newborn mice, therefore, consisted of a 1-day delay of virus replication in the liver and spleen, a 2-day delay of appearance of virus in the blood, and a suppression but no delay of virus replication in the lung and brain. These results correlate with the 1-day increase in mean survival time. The failure of ara-C therapy to reduce final mortality of mice infected with HVH appears to be directly related to the inability of the drug to persistently and effectively inhibit viral replication in target organs.

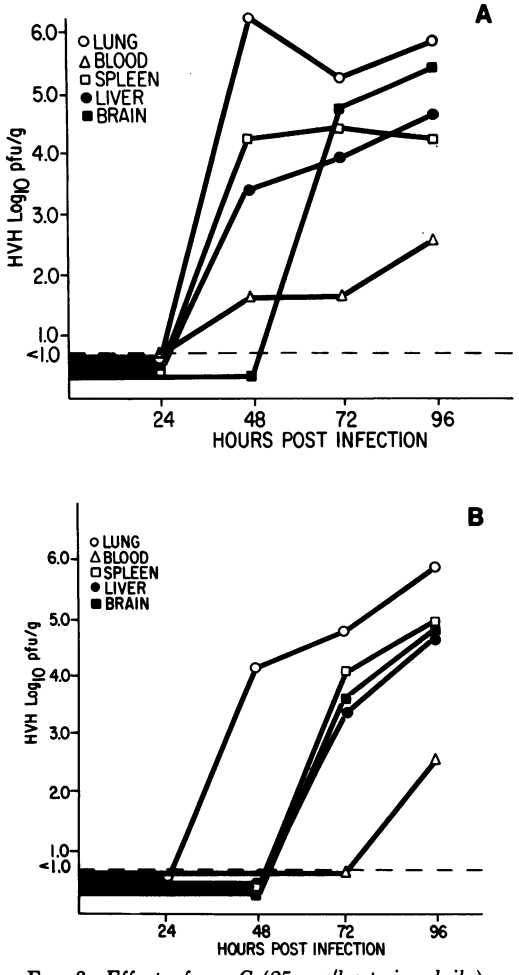

FIG. 3. *Effect of ara-C (25 mg/kg twice daily) on the pathogenesis of HVH-2 infection in newborn mice. (A) Untreated control group; (B) treated group.*

Effect of ara-A treatment on pathogenesis. Although treatment of HVH-infected newborn mice with ara-A also failed to reduce the final mortality, there was a 2-day increase in the mean survival time. These results suggested that treatment was effective, at least temporarily, in altering virus replication in target organs. To define the effects of ara-A therapy on pathogenesis of the infection, experiments similar to those described with ara-C were performed. In control animals (Fig. 4A), virus titers in the lung were 10^4 and 10^6 PFU/g of tissue at 72 and 96 h, respectively. Although virus was not detected in the blood until 96 h, both liver and spleen had titers of greater than 10^4 PFU/g by 72 h. It is likely that there were low levels of virus in the blood that were not detected at earlier time periods. Virus was first detected in the brain at 96 h (10^5 PFU/g of tissue). In treated animals (Fig. 4B), there was considerable alteration of pathogenesis. Virus was not detected in lung tissue until 96 h, a 1-day delay, and replication was also suppressed throughout the course of infection when compared to control animals. The onset of viremia was delayed 1 day (from 96 to 120 h), and viral replication in liver and spleen was delayed 2 days. In addition, there was a 2-day delay in the time that virus was first detected in the brain. Alteration of HVH pathogenesis by ara-A in this model infection was characterized by a delay and/or suppression of virus replication for 2 days. The delay and suppression of HVH replication in target organs of infected animals treated with ara-A correlates with the 2-day increase in the mean survival time.

Results from the alterations of HVH pathogenesis in newborn mice treated with ara-C or ara-A indicate that treatment was effective only for a short period of time. The virus was not eliminated from the animals, however, and viral replication, although delayed and/or sup-

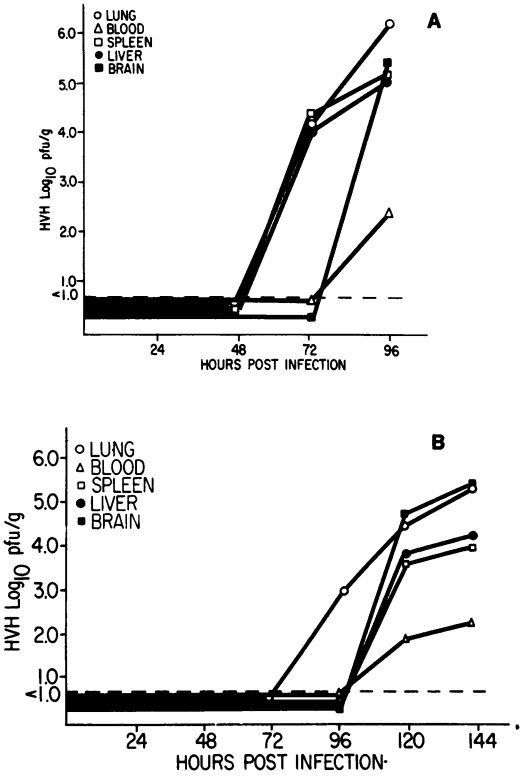

FIG. 4. *Effect of ara-A (500 mg/kg twice daily) on the pathogenesis of HVH-2 infection in newborn mice. (A) Untreated control group; (B) treated group.*

pressed, still occurred in spite of continued therapy.

Susceptibility of HVH isolated from tissues of treated mice to ara-C and ara-A. The development of resistance to IUdR after a single passage of HVH in tissue culture in the presence of the drug has been reported (5). Multiple passages of the virus in vitro in the presence of ara-C or ara-A, however, has not resulted in the development of resistance to either compound (5, 18). To determine whether HVH developed resistance to either drug during replication in newborn mice in the presence of ara-C or ara-A, the following experiment was performed. The susceptibility to ara-C and to ara-A of virus isolated from the tissues of treated as well as control mice dying in the previous experiments was compared with the stock HVH used to infect the animals. The level of susceptibility for each virus is tabulated in Table 2. No detectable difference in susceptibility to ara-C or ara-A was observed for HVH isolated from control animals, from ara-C or ara-A-treated animals, or the stock virus pool. These results indicate that HVH replicating in vivo in the presence of either antiviral agent did not develop resistance to the drug.

DISCUSSION

Both ara-C and ara-A have been reported to have anti-herpesvirus activity in a number of cell culture and experimental animal systems and have been used to treat herpesvirus infections in humans. Buthala (5) demonstrated that ara-C was active against HVH in a variety of cell culture systems including rabbit, mouse, rat, chicken, guinea pig, and human tissues. Depending on the assay system utilized, ara-C was as active or more active than IUdR against HVH in rabbit kidney cells. Resistance developed in culture against IUdR but not against ara-C. Utilizing monkey kidney cells and two human cell lines (WI-38, HeLa), Walker and co-workers (34) concluded that ara-C was more effective than IUdR against both type 1 and type 2 HVH and exhibited less cell cytotoxicity. Fiala and co-workers (10) reported that type 1 HVH strains were slightly more susceptible to ara-C than type 2 strains in WI-38 cells and in a line of fetal tonsil cells. Miller and associates (18) reported that ara-A, ara-C, and IUdR each exhibited similar levels of antiviral activity against type 1 HVH in HEP-2 cells. Resistance did not develop against low levels of ara-A on serial passage in tissue culture. When comparing the susceptibilities of HVH types 1 and 2 to ara-A, Person and co-workers (26) found that in chicken embryo fibroblasts the type 1 strains were slightly more susceptible but in WI-38 cells there were no differences in susceptibility between the type 1 and type 2 strains. In our system, there were no differences in susceptibility between type 1 and 2 strains or low-passage genital isolates in mouse embryo fibroblast, WI-38, or human foreskin fibroblast cells with either ara-C or ara-A. All strains were inhibited by lower concentrations of ara-C (0.1 to 0.6 μg/ml) than ara-A (5 to > 25 μg/ml) in the 50% plaque reduction assay in all cell culture systems. In addition, all strains of virus appeared to be more susceptible to ara-C in human than in mouse cells, but more susceptible to ara-A in mouse than in human cells. These data indicate that both ara-C and ara-A are active against both type 1 and type 2 strains of HVH in a variety of tissue culture systems and that resistance does not appear to develop to either drug in vitro.

Underwood (33) reported that ara-C was as effective as IUdR in the treatment of HVH keratitis in rabbits. Renis (28) demonstrated that ara-C treatment of rats inoculated intranasally with herpesvirus delayed the onset of paralysis and death, reduced final mortality, and delayed and suppressed viral replication in brain tissue. In contrast, Panitch and Baringer (25) demonstrated only a temporary delay in the clinical and pathological development of HVH encephalitis in rabbits infected by the

TABLE 2. *Susceptibility to ara-C and ara-A of HVH isolated from tissues of untreated control and treated mice in mouse embryo fibroblasts*

Source of virus	50% Inhibitory levels[a] (μg/ml)	
	ara-C[b]	ara-A[c]
Untreated control mice		
Lung	2.0	12.5
Liver	2.0	37
Spleen	1.0	37
Brain	2.0	9
Treated mice		
Lung	2.5	18
Liver	1.3	37
Spleen	2.0	18
Brain	2.0	18
Stock HVH pool	2.0	18

[a] Concentration of drug that reduced the virus control plaque count by 50%.

[b] ara-C susceptibility testing was performed on virus isolated from ara-C-treated and untreated control animals.

[c] ara-A susceptibility testing was performed on virus isolated from ara-A-treated and untreated control animals.

corneal scarification method and treated with ara-C. ara-A has been shown to be effective in the treatment of HVH keratitis (30) and subsequent encephalitis (29) in hamsters. Sloan and co-workers (31) reported that ara-A administered intraperitoneally, subcutaneously, or orally increased the mean survival time and reduced mortality in mice inoculated intracerebrally with herpes simplex virus. In a study comparing the effectiveness of ara-A, ara-C, and IUdR in mice inoculated intracerebrally with type 1 HVH and treated intracerebrally with these drugs, IUdR was shown to be inactive and ara-C less active than ara-A in reducing mortality (1). ara-A has also been reported to be effective in controlling the development of lesions of a cutaneous herpesvirus infection of hairless mice when ara-C and other antiviral agents were not effective (16, 17). Although both ara-C (8) and ara-A (7) have been utilized to treat serious human HVH infections, there are no published results of placebo-controlled studies that demonstrate efficacy.

In our experimental model of disseminated neonatal HVH infection, treatment with ara-C or ara-A resulted in a significant delay in the mean day of death as well as an alteration in the pathogenesis of the infection. The final mortality, however, was not reduced with either compound. In animals treated with ara-C, the appearance of virus in the liver and spleen was delayed by 1 day and in the blood by 2 days. Virus replication in lung and brain was also suppressed for a day, but not delayed, and virus titers in the tissues of ara-C-treated animals reached the same levels observed in the control animals by 72 to 96 h after infection. ara-A treatment, on the other hand, delayed the appearance of virus in the blood, lung, and brain for 1 day and in the liver and spleen for 2 days. More importantly, virus replication in the tissues of ara-A-treated animals, with the possible exception of brain tissue, appeared to be suppressed throughout the course of the infection. It appeared, therefore, that although both ara-C and ara-A markedly altered pathogenesis and significantly delayed the mean day of death, the eventual outcome of the infection was not altered. The major reason for lack of success with drug therapy appeared to be failure to completely control initial virus replication in the lung and subsequent spread to, and replication in, the central nervous system.

It is difficult to reconcile the results of successful treatment with ara-C of HVH encephalitis in rats reported by Renis (28) with the unsuccessful results reported herein in mice, or by Panitch and Baringer in rabbits (25). Since ara-C is much less toxic to rats than to mice or rabbits, the larger doses of the drug administered to rats (160 to 640 mg/kg per day for 5 days) than to mice (50 mg/kg per day for 5 days) or to rabbits (40 mg/kg per day for 3 days) may account for the differences noted. Since it has been shown that pyrimidine nucleoside deaminase plays a prominent role in breaking down ara-C to an inactive form, the facts that rat tissues do not contain this enzyme and that both mouse and rabbit tissues contain appreciable quantities might further explain the differences in treatment results (6).

The unsuccessful treatment with ara-A of newborn mice infected intranasally with HVH type 2 reported here is in contrast with the successful therapy of experimental HVH encephalitis in hamsters (29) and mice (1, 31, 32) and HVH skin infections in mice (16, 17) reported by others. In experiments reported elsewhere (24), we have shown that ara-A treatment of 6-week-old female mice inoculated intraperitoneally with HVH type 2 was highly effective in reducing final mortality, even when treatment was delayed until 48 to 72 h after virus inoculation. These results suggest that the differences in therapeutic efficacy of ara-A in the various experimental animal model systems may reflect differences in the age of the animals, the route of inoculation of virus or of drug, and/or the pathogenesis of the infection.

There are several possible explanations for the failure of ara-C and ara-A therapy to reduce final mortality in the experimental disseminated neonatal infection with HVH type 2 reported here. First, the regimen of 25 mg/kg twice daily for 5 days with ara-C or 500 mg/kg twice daily for 5 days with ara-A may have been insufficient to successfully treat the animals. This seems unlikely, however, since higher doses of the drugs or treatment with similar doses for longer periods of time increased toxicity without increasing efficacy. Second, the immunosuppressive effects of ara-C in an already compromised newborn mouse may have also contributed to the failure of the host to control the infection. Third, strains of virus which were resistant to ara-C or ara-A could have been selected during the course of therapy and could have resulted in death of the animals. Virus isolated late in the course of the infection from animals treated with either ara-C or ara-A, however, was tested and exhibited the same level of susceptibility as the original stock virus pool used to inoculate the animals. Fourth, it is possible that sufficient concentrations of the antiviral agents were not achieved in critical target organs, particularly the lung and the

central nervous system, to completely inhibit viral replication. Although we attempted to determine the levels of ara-C and ara-A in the blood and tissues of treated mice, the plaque-reduction assay utilized was not sensitive enough to detect low concentrations of the antiviral agents. Other investigators, however, have provided information concerning the pharmacology of ara-C and ara-A. ara-C, after administration to a number of different animal species is rapidly broken down to the inactive form ara-uracil and excreted. The serum half-life of tritiated ara-C in mice after parenteral administration is 20 to 30 min (2) with 85% of the radioactivity excreted in the urine over a 24-h period (9). There is no published information concerning the tissue distribution of ara-C in mice. In both in vitro and in vivo biological systems, ara-A is rapidly deaminated to the arabinofuranosylhypoxanthine analogue (3, 4). In contrast to the breakdown products of IUdR and ara-C, which have no demonstrable antiviral activity, the arabinofuranosylhypoxanthine compound has been reported to be about as effective as ara-A against herpesvirus encephalitis in the mouse (31). After intraperitoneal administration of a 1.0-mg dose of radiolabeled ara-A per kg to mice, peak levels of 30 μg/ml were noted in the blood, 25 μg/ml in the liver, 60 μg/ml in the spleen, and 50 μg/g in the kidney by 30 min after inoculation (4). No activity, other than that which could be accounted for by blood contamination, could be detected in brain tissue. Fifth, it is possible that the host defense mechanisms of newborn mice are too immature to inhibit the rapid spread and multiplication of the virus in critical target organs even in the presence of an active antiviral chemotherapeutic agent. Data from our own laboratory have confirmed the previously reported observation that newborn mice are more susceptible to herpesvirus infections than weanling or adult animals (11, 13). One might predict, therefore, that if immature host defense mechanisms were a major factor in the failure of ara-C or ara-A to successfully treat newborn mice infected intranasally with HVH type 2, treatment would be more successful in 3-week-old animals. However, in previously reported experiments (24) the treatment of 3-week-old weanling mice infected intranasally with HVH type 2 with maximally tolerated doses of ara-A produced essentially the same results as with the 5- to 7-day-old mice; the mean survival time was prolonged, but the final mortality was not reduced. In contrast, treatment with ara-A of 3-week-old mice infected intraperitoneally with HVH-2 was highly effective in protecting the animals from a lethal infection, even when the onset of therapy was delayed until 48 h after infection (unpublished data). These results suggest that the route of inoculation of virus and the pathogenesis of the infection associated with that route of inoculation may be important determinants of the success or failure of a given form of antiviral chemotherapy. The results also suggest that immature host defense mechanisms may not be a major factor contributing to the failure of ara-A or ara-C in the treatment of the HVH type 2 infection in newborn mice.

The lack of success of ara-C or ara-A therapy in this model of disseminated neonatal HVH type 2 infection appears to be due primarily to insufficient concentrations of biologically active drug in critical target organs (central nervous system and lung) to effectively inhibit viral replication. Failure of these compounds to protect newborn mice from the lethal effects of HVH type 2, however, does not preclude the possibility that they might be effective in the treatment of human disease. The experimental herpesvirus infection reported here is generally a more acute, rapidly fatal infection than that seen in most human infections. In a more slowly progressing infection, and particularly in those infections that do not have central nervous system involvement at the time therapy is initiated, ara-C or ara-A might be more effective.

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