Treatment of Herpes Simplex Virus Types 1 and 2 Encephalitis in Mice with 9- β -D-Arabinofuranosyladenine

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The susceptibility of herpes simplex type 1 and type 2 viruses to 9- β -D-arabinofuranosyladenine (ara-A) was tested in intracerebrally infected mice. Subcutaneously administered ara-A resulted in markedly equivalent and reproducible chemotherapeutic activity against both serotypes of herpes simplex viruses. Administration of ara-A by several different intermittent dosage regimens showed that change in pattern of response in terms of survivors appears to be influenced more by total amount of drug administered than by schedule or duration of drug treatment. In general, surviving drug-treated, virus-infected animals survived rechallenge with 1,000 LD₅₀ of the respective homologous virus inoculated intracerebrally on the 21st day after the initial virus inoculation. A neutralization test performed in vitro-in vivo confirmed the identity of the two distinct serotypes of herpes simplex virus employed. The data indicate that the genital form of herpes simplex virus (type 2) is as sensitive as the oral-mouth form (type 1) to the significant therapeutic activity of ara-A.

Herpes simplex virus (HSV), designated *Herpesvirus hominis* by Andrewes (2), is an ubiquitous organism which causes fever blisters and a variety of clinical disorders, including infection of the genital organs in both sexes. Genital strains of HSV are serologically and immunologically different from the mouth and eye strains (4, 9, 16). The genital form is called HSV type 2, and the serotype from the mouth and eyes is type 1. The antigenic and biological differences among HSV strains were recently reviewed by Nahmias and Dowdle (10). Moreover, the two viruses differ from each other in several biological ways (1, 10-12, 15). Recently, interest in the type 2 virus has increased because of reports suggesting its possible etiological role in causing cervical carcinogenesis (6, 13, 16, 17).

In a recent summary of a series of papers, 9- β -D-arabinofuranosyladenine (ara-A) was reported to be markedly effective in vitro and in vivo against several deoxyribonucleic acid (DNA) viruses, including HSV (19). Although in these reports (3, 7, 20-22) the strains of HSV were not designated as type 1 or 2, all had been isolated from either oral or ocular lesions and presumably represent type 1 strains. In cell culture studies, type 2 HSV was reported to be less susceptible to ara-A than type 1 HSV (S. P.

Lowry and W. E. Rawls, *Bacteriol. Proc.*, p. 171, 1969). Recent results reported by Person et al. (14) suggest that the susceptibility of the two types to ara-A varies with the cell type employed in performing the activity assessment. The present report describes the results of experiments comparing the activity of ara-A against type 1 and type 2 HSV encephalitides in mice.

MATERIALS AND METHODS

Virus strains. The REM strain of HSV type 1, isolated in our laboratory from a human oral herpetic vesicle, was passed 29 times intracerebrally (ic) in mice. Stock viral suspensions were prepared by homogenizing brains from infected moribund mice and preparing a 10% suspension in Eagle's medium or Hanks balanced salt solution (HBSS; 5) containing 5% chick or bovine serum. The PT strain of HSV (also representative of type 1) was obtained from R. L. Northrop, Presbyterian-St. Lukes Hospital, Chicago, Ill. This strain had been passaged twice in HEp-2 cell monolayers in our laboratory, and samples of supernatant fluid containing 10^{6.5} plaque-forming units (PFU) per ml were then stored frozen in glass vials at approximately -70 C until use.

The type 2 strain of HSV used in these studies was obtained from Joseph Melnick, Baylor University, who designated it as HSV-2-198. It had been isolated in monolayers of human embryonic kidney

(HEK) cells in 1968 from a vulvar lesion of a patient with genital herpes. In our laboratory, it was first propagated in HEK cells and then passed once in HEP-2 cells. Samples of the supernatant fluids were prepared in small glass vials and stored at approximately -70°C until use. The intracerebral mouse titer of this cell culture seed was 10^{-4} and contained $10^{5.7}$ PFU per ml.

For each strain of HSV employed, titers of viral inocula were calculated by the Reed and Muench method (18) from the dose-response curve for 21-day mortality of mice inoculated ic with the respective viruses. For all virus inoculations, serial dilutions of the virus were made in HBSS, and an inoculum of 32 LD₅₀ was routinely employed to infect the mice. In some mouse experiments, levels of HSV ranging from 32 through 1,000 LD₅₀ of virus (in serial one-half log₁₀ dilutions) were used to infect the animals. The animals were observed daily for death for a period of 21 days.

Mice. Random-bred albino ICR male Swiss mice (purchased from Carworth Farms, Portage, Mich.) weighing 16 to 18 g were used throughout these experiments. They were housed 10 or 20 to a cage.

Compound. Micronized (particle size, 5 μm or less) ara-A obtained from the research laboratories of Parke, Davis & Co. was used in these experiments. The micronized material was prepared from crystalline compound which had been isolated from a fermentation with strain NRRL 3238 of *Streptomyces antibioticus* (Parke, Davis & Co., Belgian Patent 708,821, 1968). Test suspensions (ara-A is only slightly soluble in water, -0.5 mg/ml at 25°C) were prepared in a sterile vehicle containing the following ingredients: sodium phosphate, monobasic anhydrous, 4.80 mg; sodium phosphate, dibasic anhydrous, 1.11 mg; phemerol chloride, recrystallized, 0.1 mg; hydrochloric acid reagent to pH 6.0; and distilled water to a volume of 1.0 ml.

Experimental methods. In these experiments, mice in groups of 10, 20, or 50 for each drug dose were inoculated ic with 0.05 ml of various doses of HSV. Ten or 20 animals were used as untreated, virus-infected controls. Initially, ara-A was administered subcutaneously (sc) three times a day for 1 day beginning 24 hr after virus inoculation. In subsequent intermittent therapy trials, the treatment schedule was modified accordingly. Each dose of ara-A used was suspended in sterile, buffered, aqueous solution and was administered in a volume of 0.5 ml. For drug toxicity controls, groups of 5 or 10 mice were inoculated ic with 0.05 ml of virus diluent only, treated sc with identical drug dosages at the same time as the test animals, and observed for 21 days.

In several experiments in which there were large numbers of surviving virus-infected, drug-treated animals, 21-day survivors were rechallenged ic with 1,000 LD₅₀ of the original HSV virus suspensions and observed for an additional 21 days for death, or survival due to immunity development.

Results were expressed as percent survivors or as increases in mean survival time after a 21-day period of daily observation of mortality. In experiments in which results were statistically analyzed, the mean

survival times of the drug-treated, virus-infected animals dying on or before day 21 were compared with the mean survival times of the virus control mice. If the P value obtained was <0.05 but >0.001 , the antiviral activity was considered questionable. If the P value was <0.001 , the drug was considered to have antiviral activity. The number of surviving infected, treated animals was compared with the number of untreated, virus control animals surviving (if any) by chi-square analysis. If the P values calculated by this method were <0.3 but >0.05 (approximate standard error), the antiviral activity of ara-A was considered questionable. Values of <0.05 , however, were considered to be significant and indicative of antiviral activity.

Rawls et al. (16) demonstrated by using neutralization kinetics in cell culture that strains of HSV can be differentiated into two groups designated types 1 and 2. Immune rabbit serum prepared against type 1 HSV neutralizes homologous virus much more efficiently than heterologous virus, whereas immune serum prepared against type 2 HSV neutralizes homologous and heterologous viruses equally. The neutralization tests in our laboratory were modified by assaying in vivo (rather than in vitro) and were performed as follows: 1 ml of type 1 or type 2 virus stock containing ca. 10^5 PFU of virus was mixed with 1 ml of 1:4 dilution of type 1 HSV antiserum. The diluent used was HBSS. The virus-serum mixture was held at 37°C , and samples were removed at intervals of 1, 4, and 8 min and diluted 1:100 and 1:1,000 in cold HBSS. Controls of virus plus HBSS were held at 37°C , and samples were taken and treated as above. The presence of unneutralized virus in the samples was determined by inoculating groups of 10 mice ic with 0.03 ml of each sample of the serum or control mixtures. Results were expressed as percent survivors after a 21-day period of daily observation of mortality.

RESULTS

The results obtained in our assay system with HEP-2 cells have confirmed the reports (14; Lowry and Rawls, Bacteriol. Proc., p. 171, 1969) that the two types of HSV differ in their susceptibility to inhibition by ara-A in cell culture. In our cell culture study, however, the 50% plaque reduction values of three HSV type 2 strains were found to be lower than those of two strains of HSV type 1. However, it seemed to be more important to determine whether this in vitro difference would reflect therapeutic efficacy in vivo. Thus, comparative animal experiments were carried out to determine whether there was a difference in sensitivity to ara-A between HSV type 1 and type 2 in vivo.

Effect of ara-A against HSV types 1 and 2. In the initial experiment, ara-A was tested against ic HSV (types 1 and 2) in mice. The drug was suspended in buffered aqueous solution and given sc three times about 2 hr apart beginning 24 hr after inoculation with 32 LD₅₀.

of virus. Results (Table 1) were essentially the same for all three viruses employed; that is, the mortality and mean survival time data indicate that ara-A, particularly at the 3,000 mg/kg dose level, was equally active against mouse infections caused by either serotype of HSV. Highly significant survivor rates were obtained with ara-A treatment against the three strains of HSV used. At the highest total drug level (3,000 mg/kg), the *P* values obtained by chi-square analysis were <0.001 . At both total drug dosages of ara-A tested (3,000 and 2,000 mg/kg), a significant increase in life span in dying animals which had been treated with ara-A was apparent. The survivors were challenged with 1,000 LD₅₀ of the respective homologous virus 21 days after previous ic inoculation with 32 LD₅₀ of virus. Practically all of these challenged survivors were immune to 1,000 LD₅₀ of virus. The one surviving untreated-inoculated control mouse died after the challenge, indicating that it had not been infected or rendered immune by the initial virus inoculation. Normal animals of the same age and weight as drug-treated survivors inoculated with the same high dose of HSV all died, displaying typical central nervous system symptoms. Since herpes encephalitis did not appear in essentially any of the challenged mice, whereas control mice of the same age and weight given the same inoculum died, it is concluded that ara-A treatment of mice controlled a virus infection sufficiently extended to stimulate solid immunity. This has been repeatedly experienced in our studies and was recently

reported elsewhere (8, 22).

Effect of virus level on activity against HSV type 2. To evaluate more thoroughly the relative effectiveness of ara-A administered to mice challenged with various levels of genital type 2 HSV, groups of 20 mice were inoculated ic with 32 through 1,000 LD₅₀ of virus (in half log₁₀ increments) 24 hr before treatment began. Ara-A was administered three times on the day after inoculation as before, with sc doses of 1,000 and 666 mg per kg per treatment. As shown in Table 2, ara-A was effective in increasing the number of survivors when the virus inoculum did not exceed 100 LD₅₀. When the size of the infecting virus dose was greater than 100 LD₅₀, the antiviral protective effect of ara-A was demonstrated by the significant increase in life span observed in dying mice compared with that seen in the inoculated-untreated control animals.

Intermittent treatment schedule. Previous animal experiences with ara-A, reported elsewhere (22), demonstrated that the effectiveness of this drug against HSV infections in mice varied with both total drug dose administered and schedule of drug treatment. Accordingly, an experiment was performed to determine the effect of intermittent therapy on increase in survivors among mice infected ic with both types HSV. Groups of mice were administered sc dosages of ara-A beginning 24 hr after ic inoculation with 32 or 1,000 LD₅₀ of type 1 or type 2 HSV. The drug was either given twice a day for one 2-day period or twice a day for four 2-day periods with intervals of 3 days between

TABLE 1. *Effect of ara-A^a on mice previously inoculated intracerebrally with 32 LD₅₀ of three strains of herpes simplex virus*

HSV strain	Total dose of ara-A (mg/kg)	Survival of uninoculated toxicity control mice at 21 days	Inoculated and treated mice				Rechallenge of 21-day survivors ^e
			Survivors at 21 days	Survivor <i>P</i> ^b	Mean survival time ^c (days)	Increase <i>P</i> ^d	
Northrop PT type 1	3,000	10/10	14/20	<0.001	7.2	<0.05	13/14
	2,000	10/10	11/20	<0.001	9.1	<0.001	11/11
	0	-	1/20		5.6		0/1
REM type 1	3,000	10/10	13/19	<0.001	10.5	<0.001	13/13
	2,000	10/10	3/20	<0.3	8.8	<0.001	3/3
	0	-	0/20		5.9		
Baylor 198 type 2	3,000	10/10	11/20		10.9	<0.001	10/11
	2,000	10/10	10/20	<0.001	12.4	<0.001	8/10
	0	-	0/20		6.2		

^a Animals were treated subcutaneously three times a day for 1 day beginning 24 hr after virus inoculation.

^b Probability (chi-square analysis) that the observed increase in survivor number was due to chance.

^c Only animals dying on or before the 21st day were considered.

^d Probability (*t* test) that the observed increase in mean survival time was due to chance.

^e On day 21, the surviving infected mice were rechallenged intracerebrally with 1,000 LD₅₀ of homologous herpes simplex virus and observed for death for an additional 21 days. Normal mice of the same weight and age were also challenged with this dose of virus, and all died within 3 to 4 days after virus challenge.

TABLE 2. *Effect of subcutaneous treatment with ara-A^a on intracerebral infections with various concentrations of herpes simplex virus (type 2 Baylor 198 strain) in Swiss mice*

Virus dose (LD ₅₀)	Total dose of ara-A (mg/kg)	Survival of uninoculated toxicity control mice at 21 days	Inoculated and treated mice			
			Survivors at 21 days	Survivor P ^b	Mean survival time ^c (days)	Increase P ^d
32	3,000	10/10	7/20	<0.05	9.0	<0.001
	2,000	10/10	5/19	<0.05	10.2	<0.001
	0	—	0/20	—	6.2	—
100	3,000	10/10	8/20	<0.05	8.6	<0.001
	2,000	10/10	3/20	<0.03	8.2	<0.001
	0	—	0/20	—	5.4	—
320	3,000	10/10	0/20	—	7.2	<0.001
	2,000	10/10	1/20	<0.3	9.0	<0.001
	0	—	0/20	—	4.4	—
1,000	3,000	10/10	0/20	—	7.8	<0.001
	2,000	10/10	0/18	—	7.2	<0.001
	0	—	0/19	—	3.6	—

^a Animals were treated subcutaneously three times a day for 1 day beginning 24 hr after virus inoculation.^b Probability (chi-square analysis) that the observed increase in survivor number was due to chance.^c Only animals dying on or before the 21st day were considered.^d Probability (*t* test) that the observed increase in mean survival time was due to chance.TABLE 3. *Effect of intermittent therapy with ara-A on mice previously inoculated intracerebrally with 32 or 1,000 LD₅₀ of herpes simplex virus (type 1 Northrop and type 2 Baylor 198 strains)*

Virus strain	Drug dosage ^a	Survival of uninoculated toxicity control mice at 21 days	Inoculated and treated mice			
			32 LD ₅₀ of virus		1,000 LD ₅₀ of virus	
			Survivors at 21 days	Mean survival time ^b (days)	Survivors at 21 days	Mean survival time ^b (days)
Northrop type 1	500 × 8 ^c	10/10	14/19	8.4	5/20	6.1
	1,500 × 2 ^d	10/10	17/20	9.7	Not done	Not done
	1,500 × 8 ^c	4/10	5/20	11.2	5/20	9.5
	0	—	4/20	6.6	0/20	3.9
Baylor 198 type 2	500 × 8 ^c	10/10	12/20	10.1	0/20	7.3
	1,500 × 2 ^d	10/10	10/20	11.3	Not done	Not done
	1,500 × 8 ^c	4/10	8/20	10.9	1/20	10.1
	0	—	0/20	7.4	0/20	4.1

^a Milligrams per kilogram per day × number of days.^b Only animals dying on or before the 21st day were considered.^c Animals were treated subcutaneously twice a day beginning 24 hr after virus inoculation on days 1, 2; 6, 7; 11, 12; and 16, 17.^d Animals were treated subcutaneously twice a day beginning 24 hr after virus inoculation on days 1 and 2 only.

each period. In the latter treatment schedule, two dosage levels of ara-A were used. The therapeutic activity of ara-A against both types of HSV in this experiment is presented in Table 3. The results indicate that ara-A was markedly effective in preventing encephalitis in mice inoculated with 32 LD₅₀ of both types of HSV. Also, equivalent numbers of survivors were obtained when a total drug dose of 3,000 mg/kg was administered over a 2-day period or when a total dose of 4,000 mg/kg was given intermittently for 8 days extending through an 18-

day period. Not too surprising (based on the results presented in Table 2) was the finding that at 21 days postinoculation few survivors remained in those groups of mice inoculated ic with 1,000 LD₅₀ of both types of HSV. A highly significant increase in life span was evident, however, in those treated, virus-infected (1,000 LD₅₀) animals which died compared with the untreated, virus-infected control mice that died.

Delayed-extended intermittent therapy. Having demonstrated marked antiviral activity

of parenterally administered intermittent doses of ara-A against both serotypes of HSV, we performed additional experiments to investigate further the response of mice infected ic with HSV (type 1 or type 2) to delayed-extended intermittent therapy with ara-A.

In the first experiment, mice, previously inoculated ic with 32 LD₅₀ of type 1 HSV, were treated sc with 1,000 mg of ara-A/kg three times a day. Mice were then randomized into groups of 20 and given intermittent doses of 500, 250, or 125 mg/kg every 48 or 72 hr over a 21-day period. The results listed in Table 4 indicate that ara-A, at the 500 and 250 mg/kg intermittent dose levels given at 48-hr intervals, was lethal for some of the toxicity control mice. However, a highly significant increase in sur-

vival was evident in the groups of mice receiving intermittent doses of 125 mg/kg during each 48- or 72-hr interval. On day 26 after virus inoculation, the surviving animals were inoculated with 1,000 LD₅₀ of homologous HSV. Most of these surviving animals survived the rechallenge. Similarly, inoculated drug control mice of the same age all died. An additional intermittent treatment experiment was performed to determine whether continued low-level treatment at 72-hr intervals after initial high (3,000 mg/kg) or low (125 mg/kg) loading doses administered 24 hr after virus inoculation would afford increased protection of mice previously inoculated ic with 32 LD₅₀ of genital HSV type 2. As shown in Table 5, when a total dose of 3,000 mg of ara-A/kg was administered as multiple doses within

TABLE 4. *Effect of intermittent therapy with ara-A in mice previously inoculated with 32 LD₅₀ of herpes simplex virus (type 1, Northrop strain)*

Intermittent dose* (mg per kg per day)	Survival of uninoculated toxicity control mice at 21 days	Inoculated and treated mice		Rechallenge of 26-day survivors ^b
		Survivors at 21 days	Mean survival time (days)	
Each 48 hr				
500	4/6	11/20 (55%)	12.1	10/11 (91%)
250	4/6	11/20 (55%)	12.0	10/11 (91%)
125	6/6	16/20 (80%)	11.7	16/16 (100%)
0		0/20	7.1	
Each 72 hr				
500	6/6	7/20 (35%)	11.5	7/7 (100%)
250	6/6	15/20 (80%)	11.2	13/15 (87%)
125	6/6	17/20 (85%)	11.6	16/17 (94%)
0		0/20	7.1	

* Animals were initially treated subcutaneously with 1,000 mg/kg three times a day for 1 day beginning 24 hr after virus inoculation and then subsequently treated subcutaneously with above intermittent doses at 48-hr (10 treatment periods) or 72-hr (6 treatment periods) intervals through day 21 after virus incubation.

^b On day 26, the surviving infected mice were rechallenged intracerebrally with 1,000 LD₅₀ of homologous HSV and observed for death for an additional 21 days. Normal mice and uninoculated toxicity control mice of the same weight and age were also challenged with this dose of virus and all died within 3 to 5 days after virus challenge.

TABLE 5. *Effect of intermittent therapy with ara-A in mice previously inoculated ic with 32 LD₅₀ of herpes simplex virus (type 2, Baylor 198 strain)*

Initial dose 24 hr after virus inoculation (mg per kg per day)	Intermittent dose at subsequent 72-hr intervals ^c (mg per kg per day)	Survival of uninoculated toxicity control mice at 21 days	Inoculated and treated mice	
			Survivors at 21 days	Mean survival time (days)
3,000 ^a	—	5/5	25/50 (50%)	11.3
3,000 ^a	125	5/5	31/48 (64%)	12.0
3,000 ^a	62.5	5/5	32/47 (68%)	11.3
125 ^b	125	5/5	17/49 (34%)	10.5
62.5 ^b	62.5	5/5	15/40 (30%)	10.3
0	0		5/50 (10%)	8.3

^a Animals were initially treated subcutaneously three times a day for 1 day beginning 24 hr after virus inoculation.

^b Animals were initially treated subcutaneously once a day for 1 day beginning 24 hr after virus inoculation.

^c Animals were administered single subcutaneous doses at 72-hr intervals subsequent to initial loading doses through day 21 after virus inoculation.

a single day, 50% survivors were obtained. When the same treatment regimen was used in addition to subsequent intermittent treatment with lower drug doses (125 or 62.5 mg/kg), an increase in response was achieved (64 or 68% survivors, respectively). On the other hand, when ara-A was initially administered in lower loading doses of 125 or 62.5 mg/kg followed by administration of similar doses intermittently at 72-hr intervals, less protection was afforded mice previously inoculated ic with type 2 HSV. Change in the pattern of response does not appear to be dependent as much upon number of divisions of total dose administered over a period of several days as upon total amount of drug administered. The intermittent studies reported herein have shown, therefore, that a minimal total drug dosage ($\geq 3,000$ mg/kg) must be administered sc (by one of several treatment schedules) to produce a significant increase in number of survivors ($\geq 40\%$). The data also indicate that low-level intermittent treatments with ara-A for several weeks may be a satisfactory therapeutic regimen for ic infections in animals.

In vitro-in vivo neutralization study. The in vivo observations reported herein show ara-A to be effective against herpes encephalitis in mice caused by the genital and oral strains of HSV. Because the type 2 strain of HSV (Baylor 198) was passaged twice in cell culture after receipt before it was used in our experimental animal studies, it occurred to us that possibly the virus had changed in some of its characteristics. To check this, a neutralization test was performed to reconfirm the identity of the Baylor 198 strain and also to establish the identity of the Northrop strain of HSV as belonging to type 1. Virus seeds of the HSV types 1 and 2 (titers of $10^{6.5}$ and $10^{5.7}$ PFU per ml, respectively) were diluted to contain 10^5 PFU/ml for use in the test. The two virus seeds, when diluted in one-half $\log_{10}$ increments and inoculated into groups of mice, gave LD_{50} values of 10^{-5} and $10^{-4.8}$, respectively, indicating that every type 1 mouse LD_{50} represented 1.3 logs of PFU and every type 2 mouse LD_{50} represented 1.2 logs of PFU. Table 6 summarizes the results of the neutralization experiment. Marked differences in mouse survival were evident depending on the serum-virus mixture inoculated into the mice. These results strongly indicate that the two strains of HSV differ markedly in their antigenic characteristics. The rabbit immune serum prepared against type 1 HSV neutralized the Northrop strain of HSV (homologous virus) much more efficiently than the Baylor strain of HSV (heterologous virus).

Rawls et al. (16) in their neutralization kinetics experiments determined the presence of surviving virus by the plaque assay technique. Our results corroborate those of these investigators, although assays of samples were performed in vivo rather than in vitro. The data clearly indicate that the Baylor HSV 198 is a type 2 strain and the Northrop HSV is a type 1 strain.

DISCUSSION

Ara-A is markedly and equally active against HSV type 1 and type 2 ic infections in mice. Statistically significant anti-HSV activity was seen in mice inoculated ic with 32 or 100 LD_{50} of virus. Virus-infected, ara-A-treated animals which survived 21 days after virus inoculation survived ic rechallenge with 1,000 LD_{50} of virus, indicating that ara-A aborted a virus infection sufficiently established to elicit host immune response. These data suggest that ara-A acts therapeutically (and not prophylactically) against both types of HSV.

When intermittent administration schedules were used, administration of equivalent doses of ara-A, for a duration of 2 days or discontinuously but at intervals over a 16-day period, was found not to be important in determining thera-

TABLE 6. Neutralization of types 1 and 2 herpes simplex virus by standard rabbit type 1 antiserum

Virus strain	Sample combination and final dilution	Sampling period (min)	Survivors at 21 days
Northrop type 1	Virus plus serum 1:100	1	3/10
		4	8/10
		8	10/10
	1:1,000	1	9/10
		4	10/10
		8	8/10
	Virus plus diluent 1:100	1	0/10
		4	0/10
		8	0/10
Baylor 198 type 2	Virus plus serum 1:100	1	0/10
		4	0/10
		8	0/10
	1:1,000	1	0/10
		4	0/10
		8	3/10
	Virus plus diluent 1:100	1	0/10
		4	0/10
		8	0/10
	1:1,000	1	0/10
		4	0/10
		8	0/10

* Virus-serum mixtures and virus-diluent (control) mixtures were assayed by intracerebral inoculation in mice. The antiserum was received through the courtesy of W. E. Rawls, Baylor University College of Medicine, Houston, Tex.

peutic response.

Ara-A has been reported to have broad-spectrum activity against DNA viruses in cell culture (7), to be effective topically against experimental herpes keratitis in hamsters (21), to protect against secondary encephalitis (20), and to possess significant therapeutic activity when given parenterally, perorally, or percutaneously to mice with experimental herpes and vaccinia encephalitis without blocking immune response (3, 22). Clinically, HSV type 1 is probably the cause of nongenital lesions such as gingivostomatitis and keratoconjunctivitis, whereas HSV type 2 is associated with genital lesions in both males and females. Also, Josey et al. (6) suggested that genital HSV may play a role in the etiology of cervical cancer, although the evidence is still largely circumstantial. However, if this speculation were to become a reality, clinical trial of ara-A in man against this disease would appear to be strongly indicated.

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