Effects of Topical Applications of Phosphonoacetate on Colonization of Mouse Trigeminal Ganglia with Herpes Simplex Virus Type 1†

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The effects of topical application of phosphonoacetic acid on the colonization of mouse trigeminal ganglia by herpes simplex virus type 1 were examined. The results showed that the extent of colonization of ganglia by virus is related to the time elapsed between virus inoculation and application of this agent. In most cases, treatment started up to 12 h after inoculation prevented invasion of ganglia by virus. When started up to 24 h after inoculation, treatment reduced and stabilized the amount of virus detectable in trigeminal ganglia during the acute phase of the ganglionic infection. Treatments started 24 h after virus inoculation had little influence on total virus accumulations in trigeminal ganglia. The data also indicate that virus titers in specimens of inoculated and treated skin sites are less affected by topical phosphonoacetic acid treatment than virus titers in ganglia. The experiments may represent a model system for testing effects of other antiviral compounds on colonization of ganglia by virus and may provide some clues regarding the pathogenic mechanism of herpes simplex virus infections.

Previous studies have shown that after peripheral inoculation, herpes simplex virus (HSV) may be detected in ganglia by 20 h postinfection (p.i.), and quantitative data have been obtained regarding the progress of virus accumulation in sensory ganglia during the early phases of the infection (1). Other experiments have shown that topical application of phosphonoacetic acid (PAA), acyclovir, or phosphonoformic acid shortly after virus inoculation can prevent in most cases the colonization of ganglia with HSV (4, 6, 7, 13, 15, 17). Compounds such as arabinosyl adenine or arabinosyl adenine monophosphate are ineffective on comparably early application (5).

There is no information, however, on the extent to which antiviral compounds can limit the amount of virus which invades the ganglia during the acute phase of infection. This information is important, since the amount of virus invading ganglia during the early phase of infection may determine the severity of the primary episode and the number of latently infected neurons and could also influence the frequency of subsequent recurrent episodes and the duration of HSV latency.

In the present study, we determined the titer of infectious virus detectable in trigeminal ganglia of mice after a series of topical applications of PAA initiated at various intervals after virus inoculation. Furthermore, we monitored on a daily basis, during and after the end of the antiviral treatment, the evolution of virus titers in trigeminal ganglia and the inoculated skin area of HSV-infected mice. The results indicate that the interval between virus inoculation and initiation of antiviral treatments has an important influence on the amount of virus which accumulates in ganglia and determines, to a large extent, the outcome of the experimental HSV infection.

MATERIALS AND METHODS

Virus. HSV type 1 (HSV-1) strain S was used in all experiments. The maintenance of this strain, the preparation

of stock virus, and the quantification of inocula have been described previously (3-7). This strain has the ability to invade the nervous system and to establish acute and latent infections in sensory ganglia.

Inoculation of mice. Female hairless mice, strain HRS/J, obtained from Jackson Laboratories, Bar Harbor, Maine, were used in the experiments at the age of 6 to 8 weeks. They were inoculated percutaneously on a triangular area of the snout by rubbing a virus suspension containing 10^6 PFU/ml into the scarified skin. With this procedure, ca. 10^4 PFU were applied on the scarified skin of each mouse.

Monitoring of infectious virus in sensory ganglia and skin specimens. At various intervals after inoculation, groups of mice were exsanguinated by heart puncture under pentobarbital sodium anesthesia. The day on which each group of mice was to be sacrificed had been randomly assigned after virus inoculation. Sensory ganglia were removed and homogenized immediately by sonication (Branson Sonifier Cell Disruptor 200). Skin specimens were removed from the inoculation area (ca. 0.25 cm^2) and homogenized by sonication. The tissue suspension was clarified by centrifugation, and its virus content was determined by a plaque assay in Vero cells.

Antiviral compounds. A 2% PAA ointment (Abbott Laboratories, North Chicago, Ill.) in a petrolatum base was used for the topical treatment of primary HSV-induced skin infections. Treatments were applied three times daily over a period of 8 h and were initiated at various time intervals after the percutaneous viral infection. Treatments were given until the sacrifice of the mice or for a maximum of 4 days.

RESULTS

Virus titers in trigeminal ganglia in relation to the initiation time of topical PAA treatment. In the first experiment, PAA applications were started 3, 24, and 48 h p.i. and in the second experiment of 3, 6, 12, and 18 h p.i. Untreated and treated mice were killed 4 days p.i., and the virus titer of ganglia homogenates was determined. The results are shown in Table 1.

Application of PAA 48 h p.i. did not influence the colonization of ganglia by virus; after 4 days the titers were essentially equal in treated and untreated mice. When appli-

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TABLE 1. Virus titers in trigeminal ganglia in relation to time of initiation of topical PAA treatment

Expt	Start of treatment (h p.i.)	Frequency of mice with virus-positive ganglia	Mean virus titer $\pm$ SD ($\log_{10}$ units) ^a
1	Untreated	10/10	4.32 $\pm$ 0.65
	3	1/10	0.30
	24	8/10	3.16 $\pm$ 0.98
	48	10/10	4.61 $\pm$ 0.43
2	Untreated	9/10	4.01 $\pm$ 0.69
	3	1/10	2.07
	6	2/10	2.78 $\pm$ 0.54
	12	0/10	
	18	6/10	3.01 $\pm$ 1.02

^a The virus titer of ganglia homogenates was determined in all mice 4 days after infection.

cations were started 3 to 12 h p.i., virus was detected in the ganglia of only 4 out of 40 treated mice. The individual titers of infectious virus in ganglia showed wide variations and were on the average about 100 times lower than the titers found in ganglia of untreated mice. Applications started at 18 or 24 h p.i. were not able to prevent the establishment of latency in a significant proportion of the mice; however, the mean virus titers 4 days p.i. were about 1/10 those in untreated mice. Overall, the data indicate that application of PAA started up to 12 h p.i. prevented the colonization of ganglia by HSV in 90% of the cases and that applications started up to 24 h p.i. reduced significantly the amount of virus which could be detected in ganglia by day 4 p.i.

Evolution of skin lesions and of virus titers in ganglia and skin specimens in treated and untreated mice. The previous experiment showed that PAA application initiated 18 h p.i. reduced the number of latently infected mice and the amount of virus detectable in ganglia 4 days p.i. Since there is a possibility that virus titers in ganglia may increase after 4 days of drug treatment, we followed the evolution of skin lesions and of virus titers in trigeminal ganglia and skin specimens during the 4-day treatment period and for another 5 days after the end of the treatment. Groups of four mice, untreated or treated 18 or 42 h p.i., were examined in parallel.

(i) Evolution of skin lesions. All untreated mice and about 80% of mice treated 42 h p.i. developed skin lesions visible by the third day p.i. (Table 2). Between 10 and 50% of mice treated 18 h p.i. presented lesions in each of the 9 days of observation. All untreated mice developed lesions of maximal intensity. The mean lesion score of mice treated 42 h p.i. was significantly lower than that observed in untreated animals. The lesions observed in mice treated 18 h p.i. were minimal. The mortality of untreated mice was two out of eight on day 8 p.i., and three out of four on day 9 p.i. One mouse treated 42 h p.i. died on day 8. None of the mice treated 18 h p.i. died over the 9-day observation period.

(ii) Virus titers in skin specimens. In untreated mice, high virus titers were detected by day 2 p.i. (Table 3). The mean titer peaked by day 5 p.i.; a relatively high titer was still present on day 9 p.i. in the only untreated surviving mouse. After day 2 p.i., all skin specimens of untreated mice were virus positive. The mean virus titers in skin specimens of mice in which PAA treatment was initiated 42 h p.i. paralleled closely those found in untreated mice, although they were about two to four times lower. Virus was detected in 24

out of 27 mice (89%) treated 42 h p.i. In mice treated with PAA 18 h p.i., virus was detected only in 14 out of 33 (42%) skin specimens. The difference in frequency of virus-positive skin specimens between mice treated 18 h p.i. and those untreated or treated 42 h p.i. was statistically highly significant ($P < 0.001$). With one exception, the titers never exceeded 10^4 PFU, as opposed to titers ranging consistently between 10^5 and 10^6 PFU found in untreated mice or in mice treated 42 h p.i. It should be noted that despite slight differences between virus titers in skin specimens of untreated mice and in mice treated 42 h p.i., the evolution of the disease was much milder in the latter than in the former group.

(iii) Virus titers in ganglia. The virus titers in ganglia of untreated mice increased steadily, peaked ($10^{4.5}$ to 10^5 PFU) by day 5 p.i., and then decreased continuously (Table 3). During the first 4 days, the rate of increase of virus in ganglia of mice treated 42 h p.i. was similar to that observed in untreated mice. However, the titer peaked by day 4 and then started to decline. The rate of decrease was slower than in untreated mice. Starting with day 4 p.i., isolated ganglia (14 out of 66) from 33 mice treated 18 h p.i. contained small amounts of virus. None contained more than 10^3 PFU, and 10 ganglia contained less than 10 PFU.

The data suggest that topical antiviral compounds can limit the amount of virus which colonizes the ganglia when the treatment is started up to 24 h p.i. Treatments initiated after 24 h do not significantly reduce virus titers in the ganglion, but promote, nevertheless, the survival of mice.

DISCUSSION

The results showed that the time at which topical antiviral treatment is initiated in percutaneous HSV-induced skin infection of mice had a definite influence on the amount of virus which accumulated in the ganglia. Treatments initiated between 3 and 12 h p.i. prevented completely colonization of ganglia by HSV. Treatments started between 18 and 24 h p.i. reduced the amount of detectable virus but did not prevent penetration of ganglia by virus.

When treatment was initiated 18 h p.i., small amounts of virus were detected in ganglia, and no trend toward an increase of virus titer was observed during and after the topical antiviral treatment. It appears that the virus titers became stabilized at levels reached before initiation of antiviral treatment. It is likely that PAA, by inhibiting virus replication at the inoculation site, also prevented further virus migration toward the ganglia. This would suggest that virus multiplication in ganglia is limited and that most of the virus originates from the skin site of the primary infection.

Untreated experimental HSV infections in mice have in most cases a fatal outcome due to the dissemination of virus in the central nervous system (1). Our results showed that a topical PAA treatment started 2 days p.i., which did not significantly affect virus titers in skin and ganglia, had nevertheless a beneficial effect on the evolution of skin

TABLE 2. Evolution of lesion scores in untreated and PAA-treated mice

Treatment ^a	Lesion score ^b at p.i. day:					
	4	5	6	7	8	9
None	1.16	2.67	3.25	3.93	4.00	4.00
PAA 42 h p.i.	0.60	1.33	1.92	2.64	3.20	2.40
PAA 18 h p.i.	0.00	0.10	0.25	0.36	0.60	0.17

^a Treatments applied three times daily for 4 days.

^b Lesion scores calculated on a scale from 0 to 4 as described in reference 7.

lesions and the survival of mice. It is therefore likely that not only the total virus accumulations in ganglia but also the continuous virus supply from the inoculation site lead eventually to central nervous system involvement and death of the mice.

The high virus titers in the skin of mice treated 42 h p.i. raise an interesting question. Antiviral treatments initiated early keep virus titers low, but those started with delay did not significantly affect the virus content of skin specimens. In fact, the evolution of virus titers in untreated mice and mice treated 42 h p.i. was very similar, being only two to three times lower in the latter.

The high virus titers detected in the skin, despite local application of a potent inhibitor of HSV replication, might be explained by the hypothesis of Stanberry et al. (14): the virus, after an initial colonization of the ganglia, returns by centrifugal axonal migration to the skin. The delay of 3 to 5 days between virus inoculation and the development of lesions would support this idea. Furthermore, it is known that, in experimental infections, virus is first detected in ganglia, and only afterward in nerves (1, 3, 8, 9, 16). The inability to detect virus in nerves during the early phases of infection might be explained by recent experiments of Lycke et al. (12), who have shown that in neuron cultures, virus is present in neuritic extension as a nonenveloped nucleocapsid. The fully enveloped virions which have been demonstrated in electron optic images by day 3 p.i. (2, 8, 10, 11) would then be virus in the process of centrifugal migration. Virions emerging from nerve endings in the deeper layers of the skin might be less affected by topical antiviral treatments and would maintain the high titers in skin specimens obtained from mice in which the treatment was initiated after the uptake and centripetal migration of the virus toward the ganglia.

The approach used in our experiments represents a model system through which the effects of other antiviral compounds and treatment modalities on the colonization of sensory ganglia by HSV can be evaluated.

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TABLE 3. HSV titers in skin and trigeminal ganglia homogenates of untreated and PAA-treated hairless mice after percutaneous orofacial infection

Specimen	Treatment ^a	Virus titer (log ₁₀ PFU ± SD) at p.i. day: ^b									% Virus positive
		2	3	4	5	6	7	8	9		
Skin	Untreated	4.99 ± 0.05 (2/4)	5.34 ± 0.04 (4/4)	5.61 ± 0.28 (4/4)	6.10 ± 0.24 (4/4)	5.74 ± 0.24 (3/3)	4.88 ± 1.18 (4/4)	4.29 ± 0.61 (4/4)	3.85 (1/1)	93 (26/28)	
	PAA 42 h p.i.	NT ^c	4.81 ± 0.43 (4/4)	5.15 ± 0.32 (3/4)	5.93 ± 0.39 (4/4)	5.72 ± 0.09 (3/3)	4.18 ± 0.86 (3/4)	3.96 ± 0.85 (3/4)	3.45 ± 1.72 (4/4)	89 (24/27)	
	PAA 18 h p.i.	1.46 ± 1.27 (3/4)	(0/4)	3.74 (1/4)	2.77 ± 1.46 (2/4)	3.67 (1/3)	2.18 ± 2.08 (2/4)	2.89 ± 2.56 (2/4)	1.58 ± 1.47 (3/6)	42 (14/33)	
Ganglia	Untreated	0.45 ± 0.25 (3/8)	2.17 ± 0.45 (8/8)	3.97 ± 0.73 (8/8)	4.75 ± 0.25 (8/8)	3.51 ± 0.32 (6/6)	2.66 ± 0.44 (8/8)	1.24 ± 0.76 (8/8)	0.15 ± 0.21 (2/2)	95 (51/56)	
	PAA 42 h p.i.	NT	2.66 ± 0.49 (8/8)	3.89 ± 0.68 (5/8)	3.72 ± 0.68 (8/8)	2.85 ± 0.67 (6/6)	1.80 ± 1.60 (6/8)	1.74 ± 1.05 (6/8)	0.94 ± 0.76 (6/8)	83 (45/54)	
	PAA 18 h p.i.	(0/8)	(0/8)	1.56 (1/8)	1.45 ± 0.94 (2/8)	0.48 (1/8)	2.32 ± 0.86 (2/8)	0.08 ± 0.15 (4/8)	0.08 ± 0.15 (4/12)	21 (14/66)	

^a Treatment applied three times daily, for a period of 4 days.

^b In brackets, number of virus-positive specimens/number of specimens examined.

^c NT, Not tested.

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