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Suppression of infectious virus spread to the liver by foscarnet following lethal infection of acyclovir-resistant herpes simplex virus type 2 in mice

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Abstract

Patients with the acquired immune deficiency syndrome (AIDS) occasionally develop hepatitis, pneumonia or esophagitis due to herpes simplex virus type 2 (HSV-2) infection. HSV hepatitis is a rare but serious complication in liver transplantation. Acyclovir-resistant HSV strains may emerge in immunocompromised patients. Following intraperitoneal inoculation, HSV-2 induces necrotizing hepatitis in mice. We studied the virus spread and mortality following intraperitoneal inoculation of HSV-2 RK (an acyclovir-resistant recombinant virus with altered thymidine kinase activity) as compared to its parent virus 8620K. Neither the 50% lethal dose (LD_{50}) nor the average survival time was significantly different between the two strains. Parenteral acyclovir treatment was found to be effective against 8620K but not RK infection. Parenteral foscarnet treatment was effective against both RK and 8620K, and also inhibited the spread of either virus to the liver, spinal cord and brain. Peroral foscarnet administration was found to prevent the virus growth in the liver.

Keywords: Acyclovir;Drug resistance;Foscarnet;Generalized infection;Herpes simplex virus

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1. Introduction

Herpes simplex virus (HSV) type 2 (HSV-2) as well as HSV type 1 (HSV-1) induces progressive, life-threatening diseases more often in immunocompromised patients than in immunocompetent patients (Whitley et al., 1984). Also, in profoundly immunocompromised patients, acyclovir (ACV)-resistant virus may emerge; thus being more life-threatening than in immunocompetent patients (Chatis and Crumpacker, 1991). The drug-resistant variants isolated most commonly have a thymidine kinase (TK)-deficient phenotype (Collins and Darby, 1991). Although the viral TK contributes to the virulence of HSV, at least some HSV mutants with low TK activity retain neurovirulence (Field and Wildy, 1978; Sakuma et al., 1988; Erlich et al., 1989). Recently Tanaka et al. (1993) prepared ACV-resistant recombinants containing an altered TK gene from HSV-2 YS-4 C-1 and compared the neurovirulence of one of the recombinants, RK, with its ACV-sensitive parent virus (8620K).

Herpes simplex hepatitis is a rare but serious complication in organ transplantation (Kusne et al., 1991). Patients with the acquired immune deficiency syndrome (AIDS) occasionally develop hepatitis, pneumonia and/or esophagitis due to HSV-2 (Safrin, 1992). Foscarnet (PFA, phosphonoformate) (Helgstrand et al., 1978) is a viral DNA polymerase inhibitor (Öberg, 1983; Crumpacker, 1992) and has been used in the treatment of ACV-resistant HSV infections (Chatis et al., 1989).

An intraperitoneal (ip) inoculation with HSV-2 in mice induces necrotizing hepatitis (Mogensen, 1977), and can thus be considered as a model for HSV infection with an increased virus load, as seen in immunocompromised patients (Anuras and Summers, 1976). In this study, we first determined the pathogenicity of 8620K and RK following ip inoculation in mice. Infectious virus was found not only in the spleen, liver and brain, but also in the esophagus and lung. Secondly, we studied the effects of PFA on both survival and virus spread after a lethal ip challenge of RK, 8620K or YS-4 C-1. We noted an increased survival and/or suppression of virus propagation in the infected mice treated with PFA via the ip route. Peroral (po) administration of PFA via the drinking water was found to suppress virus growth in the liver.

2. Materials and methods

2.1. Mice

Four-week old C3H/HeN mice were obtained from the Laboratory Animal Center of our University and were inoculated at 5 weeks of age.

2.2. Drugs

PFA (phosphonoformic acid, trisodium salt), adenine 1-β-D-arabinofuranoside (Ara-A), and thymine 1-β-D-arabinofuranoside (Ara-T) were purchased from Sigma Chemical Co. (St. Louis, MO); ACV was from Nippon Wellcome Co. (Osaka, Japan); phosphonoacetic acid (PAA) was from Nacalai Tesque, Co. (Kyoto, Japan).

Table 1
HSV-2 strains used and their LD_{50} for ip inoculation

Virus strain	TK activity	LD_{50} (PFU)	Reference
YS-4 C-1	altered	3.2×10^2	Sakuma et al., 1988
YS-4 C-2	wild type	1.0×10^3	Sakuma et al., 1988
8620K	wild type	6.0×10^2	Sakuma et al., 1988
RK	altered	5.6×10^2	Tanaka et al., 1993
A-3	deficient	$>1\times10^6$	Sakuma et al., 1988

For the in vivo ip administration, either PFA or ACV was dissolved in phosphate-buffered saline (PBS, pH 7.2) and approximately 0.1 ml per mouse (depending on the weight of the individual animal) per injection was given ip every 12 h for a total of 20 times beginning 3 h post-inoculation unless otherwise specified. The control mice received an injection of PBS instead of the drug. For the in vivo po administration, PFA was dissolved in distilled water and administered ad libitum via the drinking water.

2.3. Viruses

The viruses used are listed on Table 1. HSV-2 YS-4 C-1 and C-2 (Sakuma et al., 1988) were isolated by plaque cloning of a unique clinical isolate YS-4 (Tasaki et al., 1975). The HSV-2 strain 8620K is an isolate from a genital herpetic lesion (Sakuma et al., 1988; Tanaka et al., 1993). A-3 is a TK-deficient mutant of 8620K (Sakuma et al., 1988) and showed the highest resistance against ACV. YS-4 C-2 and 8620K showed a wild-type TK phenotype (Sakuma et al., 1988), whereas YS-4 C-1 had an altered TK with a single amino acid change at position 182 from serine to asparagine (Tanaka et al., 1993). A recombinant strain RK was derived from 8620K and carried the mutant TK gene of YS-4 C-1 (Tanaka et al., 1993).

The titration of the infectivity of each virus stock was done on Vero cells and was expressed as plaque-forming units (PFU) per milliliter. For the ip inoculation of the virus, the virus fluid was diluted in Eagle's minimal essential medium containing 2% calf serum, and 0.1 ml per mouse was inoculated. To obtain a 50% lethal dose (LD_{50}) (Reed and Muench, 1938), 10-fold dilution of the virus fluid was made and for each dilution, 5 or 6 mice were inoculated and observed for as long as 28 days.

2.4. Plaque-reduction assay

The sensitivity of HSV to the drugs was assessed by a plaque-reduction assay using Vero cells (Sakuma et al., 1988). After adsorption for 1 h, the infected cells were overlaid with a medium containing 1% methylcellulose and various concentrations of an antiviral drug and were further incubated for 2 days. The 50% effective dose (ED_{50}) corresponds to the drug dose achieving 50% plaque reduction.

2.5. Determination of virus titers in various tissues

The mice were killed at various times after the virus inoculation and tissue samples from the liver, kidneys, adrenal glands, spleen, brain, spinal cord, lung and esophagus

Table 2
In vitro sensitivity of HSV-2 8620K, RK, A-3 and YS-4 C-1 to ACV, PFA, PAA, Ara-A and Ara-T

HSV-2 strain	Drug sensitivity (ED_{50}; μg/ml)				
	ACV	PFA	PAA	Ara-A	Ara-T
8620K	0.24 ± 0.14	54.8 ± 12.4	12.4 ± 0.79	3.00 ± 0.9	3.31 ± 0.96
RK	18.6 ± 2.50	53.7 ± 6.7	11.0 ± 1.89	2.91 ± 1.0	> 100
A-3	21.3 ± 1.20	50.1 ± 9.0	13.4 ± 1.23	3.00 ± 1.0	> 100
YS-4 C-1	12.3 ± 1.25	53.6 ± 8.4	10.8 ± 0.86	3.00 ± 0.9	> 100
YS-4 C-2	1.0 ± 0.20	53.0 ± 8.0	11.0 ± 0.90	Not done	Not done

Results represent mean value (± S.D.) for three separate experiments except for Ara-A (two separate experiments).

were collected and stored at −70°C until assayed. The mice were killed at least 5 h following the final ip drug administration. Three mice per point were examined. The tissues to be titered were thawed and homogenized in PBS in either a Teflon homogenizer or with a mortar and pestle with quartz sand (Minagawa et al., 1988). The homogenized tissues were centrifuged at 2,000 rev/min for 10 min and a plaque assay of the supernatants either with or without serial dilution was performed on Vero cell monolayers in 24-well plates (Becton–Dickinson Labware, Lincoln Park, NJ). The titers of the infectious virus were expressed by PFU per tissue. The virus titers of tissues from the animals inoculated with different viruses were compared by Student's *t*-test. In addition, several isolates from the animal brain were propagated and their drug sensitivity was determined.

3. Results

3.1. In vitro sensitivity of HSV-2 strains to ACV, PFA, PAA, Ara-A and Ara-T

As shown in Table 2, RK, A-3 and YS-4 C-1 were more resistant to ACV and Ara-T than 8620K. A-3 was more resistant to ACV than YS-4 C-1, but A-3 was not significantly more resistant than RK. There was no significant difference among the ED_{50} of the tested virus strains against Ara-A, PFA or PAA.

3.2. LD_{50} of HSV-2 8620K and its derivatives RK and A-3 as compared to YS-4 C-1 and C-2

As shown in Table 1, the pathogenicity of 8620K shown by the LD_{50} following ip inoculation was retained in RK and was not significantly different from that of YS-4 C-1

Fig. 1. The tissue virus growth following an ip inoculation with HSV-2 8260K (○), RK (×) or YS-4 C-1 (△). The mice inoculated with 10 LD_{50} of either virus were killed on various days pi. The infectious virus titers of the liver, spleen, kidney, adrenal glands, spinal cord, brain, lungs and esophagus were determined. The unmarked horizontal line shows the detection limit. The asterisk (*) indicates the significantly different tissue virus titers ($p < 0.05$)

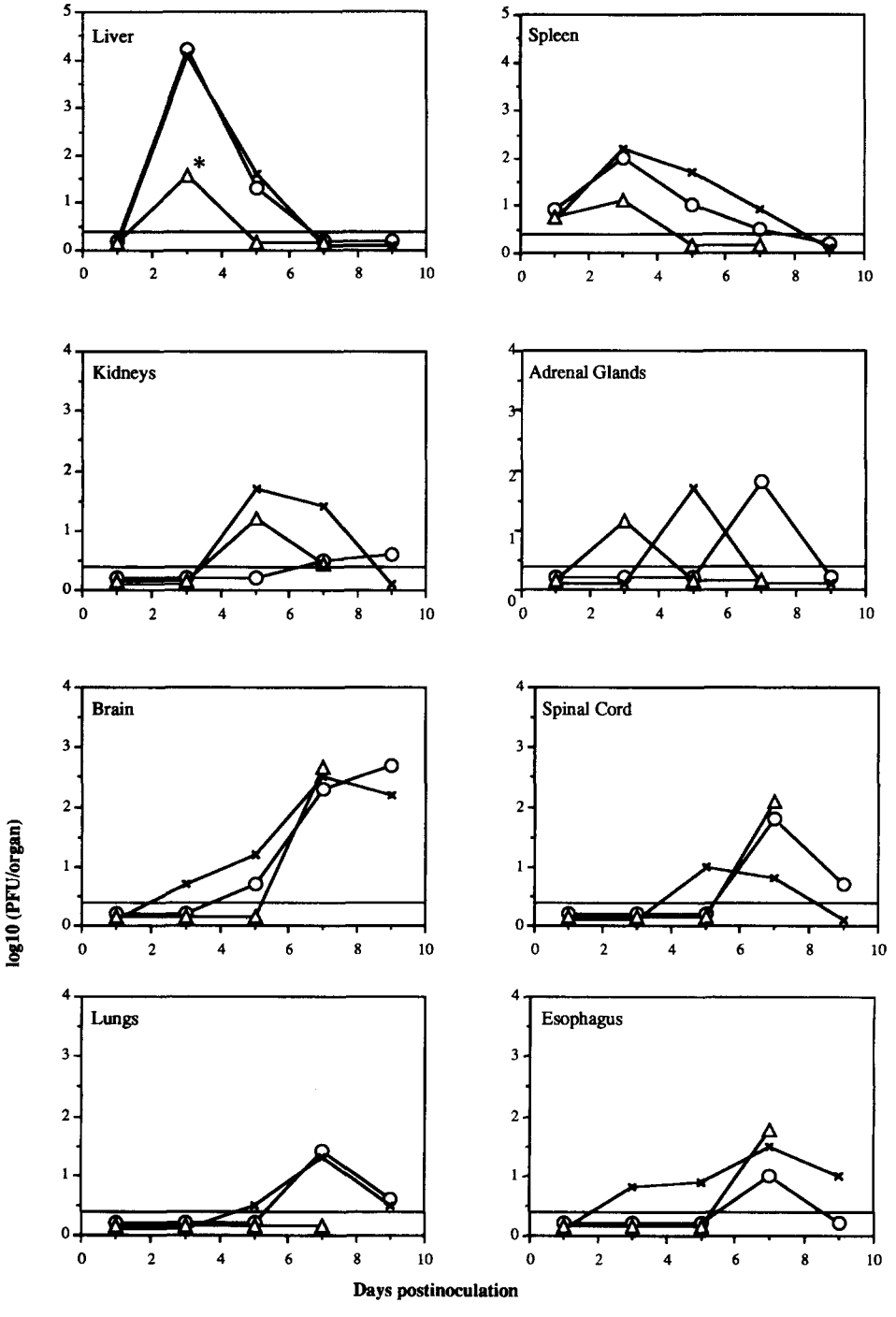
Liver
Spleen
Kidneys
Adrenal Glands
Brain
Spinal Cord
Lungs
Esophagus
log10 (PFU/organ)
Days postinoculation

Table 3
Effects of ACV and PFA on lethal ip inoculation with HSV-2 8620K or RK

Virus	Drug [a]	Route of drug administration	No. mice dead/total	Survival time (mean days ± S.D.)
8620K	PBS	ip	9/11 (82%)	7.9 ± 1.9
RK	PBS	ip	9/10 (90%)	7.9 ± 1.2
8620K	ACV	ip	2/10 (20%) [b]	12.0 ± 1.0
RK	ACV	ip	9/10 (90%)	8.7 ± 2.1
8620K	PFA	ip	1/10 (10%) [b]	10
RK	PFA	ip	0/10 (0%) [b]	–
8620K	PFA	po	10/10 (100%)	9.3 ± 2.2
RK	PFA	po	10/10 (100%)	9.3 ± 0.9

[a] Drug administration was initiated at 3 h pi. The doses were 25 mg/kg/d for ACV, 200 mg/kg/d for ip PFA and 2 mg/ml for po PFA (via drinking water).
[b] A significant difference from the control (PBS) group (Chi-square test; $p < 0.05$).

or YS-4 C-2. In contrast, A-3 significantly lost its virulence following ip inoculation. In further experiments, 10 LD_{50} was used as the standard inoculation dose.

3.3. Systemic virus spread following ip inoculation of HSV-2

As shown in Fig. 1, infectious virus first appeared in the spleen as early as 1 day post-infection (pi). At 3 days pi, the liver of the RK-, 8620K- or C-1-inoculated mice, the brain and esophagus of the RK-inoculated mice, and the adrenal glands of the C-1-inoculated mice became positive for the infectious virus. At 5 days pi, the kidneys, the adrenal glands, the spinal cord and the lungs of RK-inoculated mice, the brain of 8620K-inoculated mice and the kidneys of C-1-inoculated mice became positive for the infectious virus. By 7 days pi, 8620K had reached the kidneys, adrenal glands, the spinal cord, the lungs and the esophagus, and YS-4 C-1 had reached the brain, the spinal cord and the esophagus. None of the C-1-inoculated and PBS-treated mice survived until 9 days pi.

3.4. In vivo effects of PFA and ACV following lethal ip inoculation of either HSV-2 8620K, RK or YS-4 C-1

As shown in Table 3, ip administration of ACV was effective for the 8620K-inoculated mice but ineffective in the RK- or YS-4 C-1-inoculated mice. When the ACV dose was increased to 100 mg/kg per day, not only the virus-infected mice but also 4 of the 5 uninfected control mice died (data not included in Table 3). The ip administration of PFA was equally effective for both the 8620K- and RK-inoculated mice. PFA administration by the ip route initiated at 15 h pi resulted in 80% mortality (4 out of 5 mice died) following either 8620K or RK inoculation, while ip PFA initiated at 27 h pi resulted in 100% mortality. Administration of PFA (po, 5 mg/ml) was neither life-saving nor able to prolong the survival time.

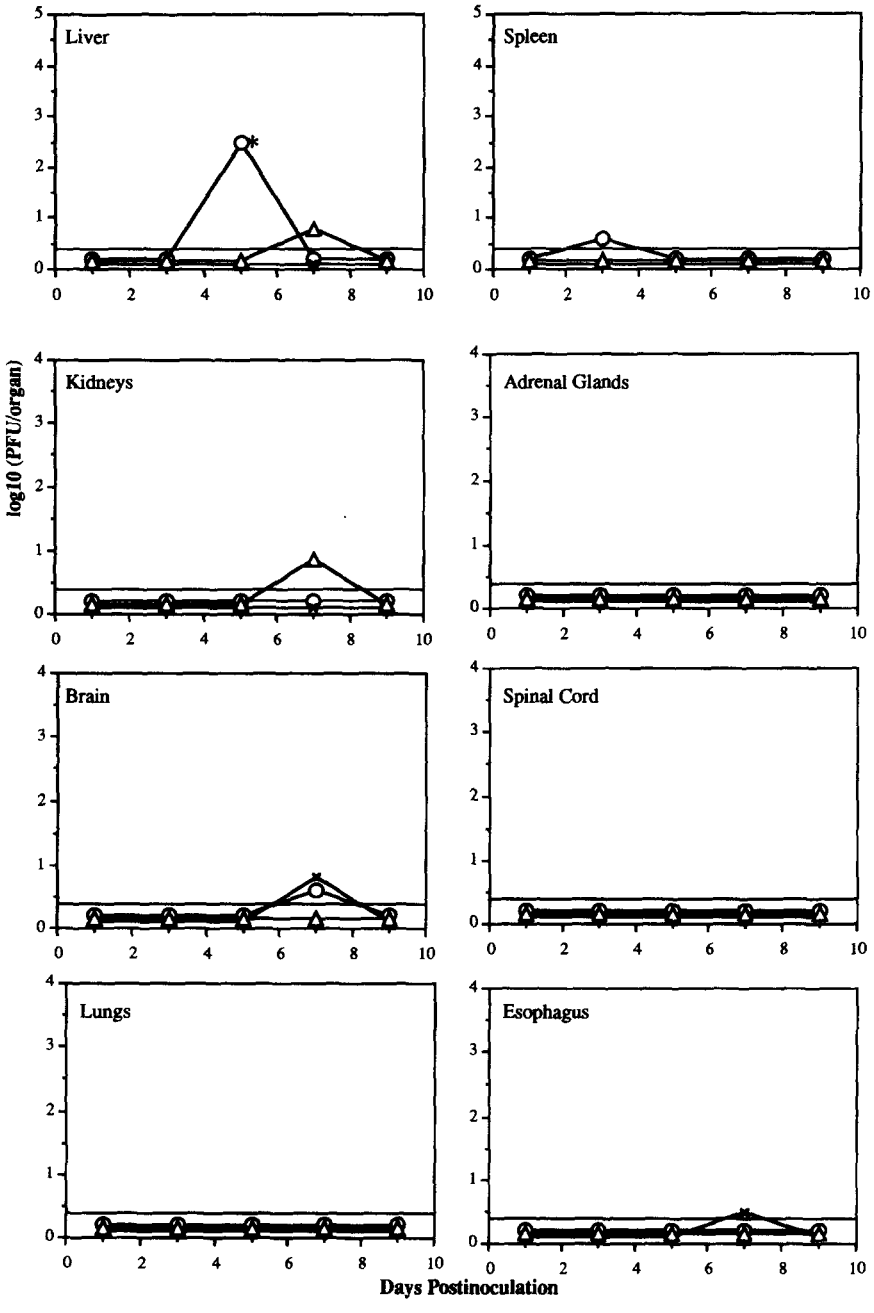

Fig. 2. The effects of ip PFA injection on the tissue virus growth following ip inoculation with either HSV-2 8620K (○), RK (×) or YS-4 C-1 (△). The mice were inoculated in the same manner as in Fig. 1 and were injected ip with 200 mg/kg of PFA per day beginning at 3 h pi, and were then killed at various days pi. Their tissue virus titers were determined in the same manner as in Fig. 1.

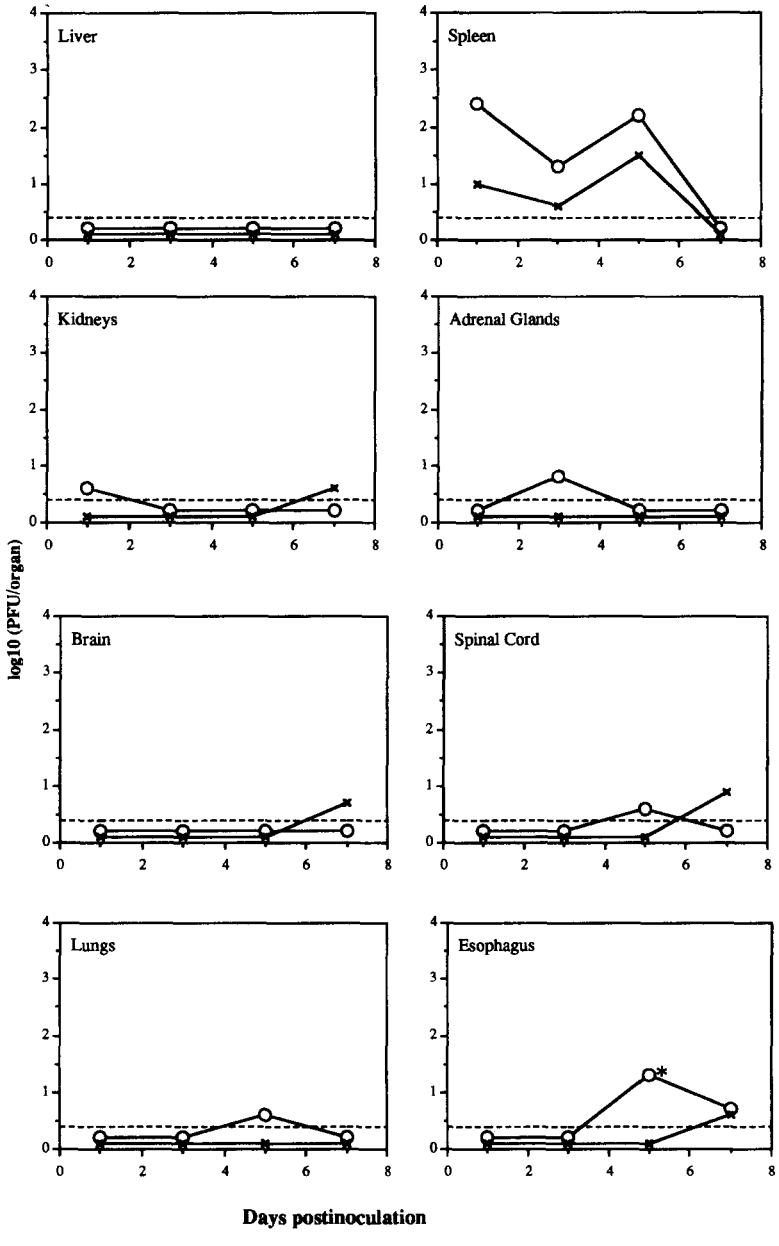

Fig. 3. The effects of po PFA administration on the tissue virus growth following an ip inoculation with either HSV-2 8620K (○) or RK (×). The mice were inoculated in the same manner as in Fig. 1 and given 2 mg/ml of PFA in their drinking water beginning 3 h pi. They were killed on various days pi. Their tissues virus titers were determined in the same way as in Fig. 1. The dashed lines show the detection limit.

3.5. Effects of in vivo administration of PFA on the tissue virus growth following ip inoculation of HSV-2

Fig. 2 shows the effects of ip administration of PFA. In all the 8620K-, YS-4 C-1-and RK-inoculated mice, systemic virus growth was markedly suppressed.

Fig. 3 shows the effects of po administration of PFA initiated 3 h pi. Virus growth in the liver was completely inhibited. However, virus growth in the spleen was not substantially affected by the drug, and the virus eventually reached the brain and other distant organs, i.e., the lungs and the esophagus, and thus the mortality was not reduced.

3.6. Drug sensitivity of the virus isolated from the brains of infected mice

As shown in Table 4, virus isolates from brain (YSB1 indicates an HSV-2 isolate from YS-4 C-1-inoculated mouse brain) retained the drug sensitivity of the inoculum virus. RKB1, RKB2, and RKB3 were isolated from different RK-inoculated mouse brains and were relatively insensitive to ACV compared to 8620KB1 and 8620KB2, which were isolated from 8620K-inoculated mouse brains.

4. Discussion

In addition to serious anorectal mucocutaneous infections, HSV-2 is also known to induce hepatitis, pneumonitis and esophagitis in AIDS patients (Safrin, 1992) and organ-transplant recipients (Kusne et al., 1991). From those patients, ACV-resistant HSV-2 has been isolated (Chatis et al., 1989; Collins and Darby, 1991; Safrin, 1992). In this study we first examined the in vitro sensitivity of HSV-2 8620K and its mutants RK and A-3 together with the other parent strain YS-4 C-1 to ACV, PFA, PAA, Ara-A and Ara-T. RK, A-3 and YS-4 C-1 were ACV-resistant but sensitive to PAA and PFA, which indicated that these were TK-mutants and not DNA polymerase mutants.

Table 4
Effects of ACV, PFA and PAA on plaque formation by different HSV-2 strains recovered from infected animal brains

HSV-2 strain [a]	Drug sensitivity (ED^{50}; $\mu g/ml$)		
	ACV	PFA	PAA
YSB1	11.5 ± 2.5	40.0 ± 10.0	10.7 ± 0.3
RKB1	13.4 ± 1.4	38.0 ± 9.2	13.9 ± 0.9
RKB2	13.5 ± 1.3	40.7 ± 9.2	13.5 ± 0.5
RKB3	13.5 ± 2.1	37.0 ± 10.2	10.3 ± 0.3
8620KB1	0.2 ± 0.1	41.0 ± 10.0	10.8 ± 0.4
8620KB2	0.2 ± 0.1	40.0 ± 11.0	12.5 ± 1.5

Results represent mean value (± S.D.) for two separate experiments.
[a] The virus strains were named after the original inoculum and the site of isolation, e.g., YSB1 was isolated from a YS-4 C-1-inoculated mouse brain.

We next noticed that the systemic spread of infectious virus following ip inoculation with HSV-2 was similar to that in weanling mice following intranasal inoculation, except that with ip inoculation, the infectious virus could also be recovered from the liver which was free from the virus following intranasal inoculation (Kern et al., 1986). It was evident that, compared to its parent virus 8620K, the alteration in TK did not diminish the ability of RK to proliferate and spread either via the hematogenous route or via the neural route. As shown in Fig. 1, RK reached the spinal cord, esophagus and even brain earlier than 8620K or YS-4 C-1, but the survival time of infected mice did not seem to correlate with the early virus detection. Therefore, RK was not more virulent than 8620K or YS-4 C-1. Drug sensitivity study shown in Table 4 indicated that the virus grown in the brains of RK-inoculated mice had indeed reduced sensitivity to ACV and was not a contaminant/revertant of the parent wild-type virus.

Then we determined the effects of ACV and PFA on the survival of mice inoculated with a lethal dose of HSV-2. As expected, ACV was effective in the 8620K-inoculated mice but was completely ineffective in the RK-inoculated mice. The death of mice given 100 mg/kg of ACV per day possibly was caused by the toxicity of the drug. On the other hand, PFA was both effective, as reported previously (Kern et al., 1978), and life-saving for the RK-inoculated mice and the 8620K-inoculated mice, if the systemic administration was carried out sufficiently early. We further examined the virus spread in these mice and found that PFA efficiently suppressed the virus spread from the intraperitoneal organs (i.e., spleen, liver) to the brain, lung and esophagus. The po administration partially inhibited the virus spread. It is also considered important to note that the virus growth in the liver was completely inhibited by the PFA if administered through the drinking water.

There are certain limitations when applying the results obtained from experimental animals to human diseases. Since there are fewer reports of DNA polymerase mutants (Sacks et al., 1989) than TK mutants, the results of this study might thus justify the prophylactic administration of foscarnet in immunocompromised patients who are at risk of HSV infection, especially for hepatitis, if the appropriate monitoring of adverse effects is available (Jacobson, 1992).

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