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Efficacy of Acyclovir Against Mouse Cytomegalovirus In Vivo

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Acyclovir reduced mortality and organ virus titers in mice inoculated intraperitoneally with 10 50% lethal doses of mouse cytomegalovirus. This susceptibility to acyclovir of a herpesvirus which lacks thymidine kinase is surprising. Alternative phosphorylating enzymes may account for this susceptibility.

Acyclovir [ACV; acycloguanosine, 9-(2-hydroxyethoxymethyl)guanine] is a purine analog which has demonstrable inhibitory activity against several members of the herpesvirus group, including herpes simplex virus (HSV) types 1 and 2, varicella-zoster virus, monkey B virus, and Epstein-Barr virus (1, 2, 4, 7, 17). Except for Epstein-Barr virus, these viruses share the property of expressing a virus-specified thymidine kinase that converts ACV to acycloguanosine monophosphate, which is further phosphorylated by host cell guanosine monophosphate kinase and other kinases (13) to its active form, acycloguanosine triphosphate, which inhibits the viral deoxyribonucleic acid (DNA) polymerase (7, 9, 10). Studies of HSV infections in mice treated with ACV have shown reduction in morbidity, mortality, and organ virus titers (8, 12, 16). We have found the in vitro susceptibility of mouse cytomegalovirus (MCMV) to ACV to be comparable to that reported for HSV types 1 and 2 and varicella-zoster virus (Burns et al., unpublished data). This was surprising since the Smith strain of MCMV is a herpesvirus which does not express a virus-specified thymidine kinase (6, 14; Burns et al., unpublished data). In this report, we extended the observation of in vitro activity to examine the activity of ACV in vivo against MCMV and found it to protect mice from lethal MCMV infection and to substantially reduce virus titers in visceral organs.

The Smith strain of MCMV was obtained from the American Type Culture Collection, Bethesda, Md. (ATCC VR-194). Virus pools for in vivo studies were prepared by intraperitoneal inoculation of weanling ICR mice (Flow Laboratories, McLean, Va.). At 3 to 4 weeks after inoculation, salivary glands were harvested, and a 10% (wt/vol) homogenate prepared in phosphate-buffered saline with 10% newborn calf serum and 35 μg of gentamicin per ml. After clarification by centrifugation at 250 × g for 5 min, aliquots were stored at −90°C until use.

Mouse embryo fibroblast cultures were prepared from 18-day-old ICR mouse embryos by overnight trypsinization at 4°C. The cells, suspended in minimal essential medium supplemented with 10% newborn calf serum and gentamicin (35 μg/ml), were seeded into 75-cm^2 flasks (Costar, Cambridge, Mass.), and grown to confluency at 37°C in 5% CO_2. Secondary mouse embryo fibroblast cultures for MCMV assay were prepared by trypsinization and by seeding 200,000 cells into 16-mm wells (Costar TC24) and were incubated in minimal essential medium supplemented with 10% newborn calf serum and gentamicin until confluent.

ACV was generously supplied as a lyophilized powder by the Burroughs Wellcome Co., Research Triangle Park, N.C. A 10^{-2} M solution in distilled water was passed through a 0.2-μm filter and diluted in medium to the appropriate concentration. Solutions were prepared fresh for each inoculation.

Adult female A/J mice (Jackson Laboratories, Bar Harbor, Maine) 7 to 18 weeks old were challenged intraperitoneally with 10 50% lethal doses of MCMV. After 4 h, treatment with ACV (or saline for controls) was initiated subcutaneously in 0.1-ml volumes. Treatment was given twice daily at an interval of 8 h for the times indicated in doses of 30, 45, or 60 mg/kg, and mice were observed for 3 weeks. Lethally infected mice died 5 to 8 days after infection. ACV given at 30 mg/kg twice daily for six doses protected few mice from death (Table 1). In contrast, ACV given twice daily at 60 mg/kg for 6 doses or 45 mg/kg for 10 doses conferred protection.

Mice infected with 10 50% lethal doses were treated twice daily for 3 days with 60 mg of ACV per kg or with 0.9% saline (controls). Groups of three control and three ACV-treated mice were sacrificed on 2, 4, 7, 10, and 18 days after infection. Samples of the liver, spleen, salivary gland, lungs, and kidneys were weighed and homogenized as a 2.5 or 10% suspension in Hanks bal-

TABLE 1. *Effect of ACV on survival after lethal infection with MCMV*

ACV dose (mg/kg)	Schedule	Treatment group[a]	No. surviving/ total no. of mice
30	Twice daily for 3 days	MCMV + saline	0/8
		ACV	8/8
		MCMV + ACV	2/9
60	Twice daily for 3 days	MCMV + saline	0/8
		ACV	7/7
		MCMV + ACV	6/6
45	Twice daily for 5 days	MCMV + saline	0/8
		ACV	10/10
		MCMV + ACV	12/12

[a] Mice were inoculated intraperitoneally with 10 50% lethal doses of MCMV as indicated. Treatment with subcutaneously administered saline or ACV was initiated 4 h after infection.

anced salt solution. Blood specimens, obtained from the retroorbital plexus, were pooled and homogenized as 10% suspensions in Hanks balanced salt solution. The organ samples were frozen at −90°C until assay. Tissue homogenates were serially 10-fold diluted, and 0.1-ml aliquots were adsorbed in quadruplicate onto confluent mouse embryo fibroblast monolayers in TC24 (Costar) wells. After 1 h, the inocula were aspirated, and the cultures were overlaid with 0.9% methylcellulose with minimal essential medium, 5% newborn calf serum, 15 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer, and gentamicin and incubated at 37°C in 5% CO_2. After 6 days, the overlays were removed, and the monolayers were fixed in absolute ethanol and stained with 0.5% methylene blue. Plaques were counted, and the results were averaged and expressed as plaque-forming units per gram of tissue. The first sacrifice of mice (2 days after infection) was performed 16 h after the preceding dose of ACV. Reconstruction experiments showed that there was no reduction in recovery of stock virus added to liver homogenates of ACV-treated mice compared with placebo-treated mice, indicating insufficient residual ACV to affect our results. Figure 1 indicates virus titers of organ homogenates from control and ACV-treated mice at various intervals after infection. Virus titers in the liver and spleen rose rapidly, exceeding 10^4 plaque-forming units per g within 2 days. In mice treated with ACV, a 1,000-fold reduction of titers in the liver, pancreas, and spleen was observed on days 2 and 4, and a 100-fold reduction of virus titer in the kidneys was seen on day 4. At later intervals, no virus was detected in the liver, whereas virus persisted in the spleen and kidneys. High virus titers developed in the salivary glands of ACV-treated mice after cessation of drug treatment. Viremia was delayed (appearing at day 7 rather than day 4) in ACV-treated mice and was 10-fold less than that in control mice.

As shown by the organ virus titer data, efficacy was achieved not by eradication of virus but rather through reduction in virus titer with the residual infection presumably controlled by host defenses. The establishment of high-titer virus in the salivary gland after cessation of treatment suggests that the establishment of latency or persistent infection was probably not affected. These results are similar to the efficacy studies of ACV in HSV infections of mice. Normal mice inoculated with HSV into the skin experienced less inflammatory local changes and decreased virus titers with ACV treatment (8). Hairless mice inoculated with HSV into the skin also experienced less severe local inflammation and reduced mortality with ACV treatment (12). In a mouse model of HSV encephalitis, ACV decreased mortality, brain virus titers, and weight loss (16). Also, if ACV treatments were started early enough and continued sufficiently long, the establishment of latent infection of dorsal root ganglia after skin inoculation was reduced. Thus, a more prolonged schedule of ACV in our MCMV model may have resulted in reduction of a persistent salivary gland infection. In perspective, although cytosine arabinoside, 5-iodo-2′-deoxyuridine, vidarabine, and phosphonoacetic acid have all been shown to have in vitro activity against MCMV, only phosphonoacetic acid has demonstrated significant in vivo activity (11, 15).

The basis for ACV activity has been well established for HSV type 1 (7, 9, 10, 17). The virus-specified thymidine kinase is necessary for phosphorylation of ACV to the monophosphate. The cellular thymidine kinase has little affinity for ACV, and little phosphorylated ACV is found in uninfected cells. The monophosphate is further phosphorylated to the triphosphate, and ACV in this form is inhibitory to the viral DNA polymerase. It can also act as a substrate for the DNA polymerase, and its incorporation into viral DNA may result in chain termination (7, 9). Viruses which lack thymidine kinase, and vaccinia, which has a thymidine kinase that does not phosphorylate ACV, are insensitive to ACV (10, 17). The importance of the virus-specified thymidine kinase for activity against HSV type 1 has been further emphasized by the production of ACV-resistant mutants, the vast majority of which have been found to be defective in viral thymidine kinase production (3, 18).

The Smith strain of MCMV lacks a virus-specified thymidine kinase (6, 14), and, thus, its

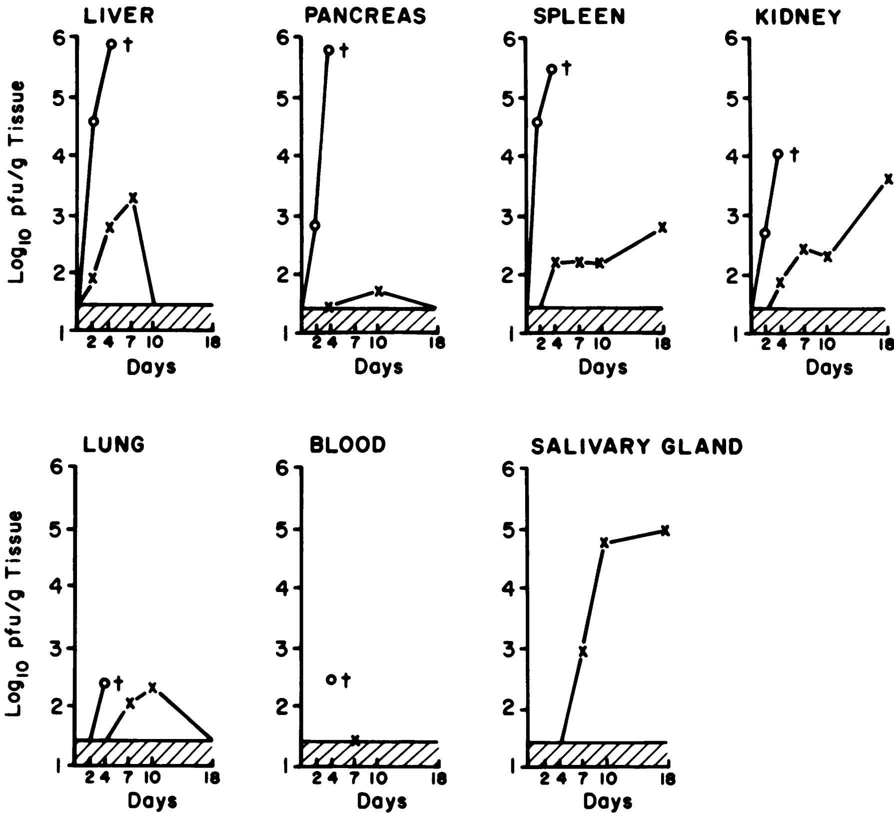

FIG. 1. *Effect of ACV on organ virus titers after lethal infection with MCMV. Mice were given saline* (O) *or 60 mg of ACV per kg* (×) *twice daily for six doses beginning 4 h after challenge with 10 50% lethal doses of MCMV given intraperitoneally. All saline-treated mice died before day 7* (†). *The hatched portion of each graph indicates the threshold of detection (25 plaque-forming units [pfu] per g of tissue).*

susceptibility to ACV is surprising. We have found that the in vitro susceptibility of MCMV to ACV is comparable to those reported for HSV types 1 and 2 and varicella-zoster virus, and we found no dependence of the antiviral activity on a thymidine kinase pathway (Burns et al., unpublished data). Similarly, no viral thymidine kinase has been identified in Epstein-Barr virus infections, yet the virus is susceptible in vitro to ACV. The Epstein-Barr virus DNA polymerase is exquisitely sensitive to acycloguanosine triphosphate and may be competitively inhibited by small amounts produced by cellular thymidine kinase (2, 5). Alternatively, Epstein-Barr virus and MCMV may specify other nucleoside kinases capable of phosphorylating ACV at a low level. Studies investigating this latter possibility are under way.

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