Acyclovir Treatment of Experimental Simian Varicella Infection of Monkeys†

KENNETH F. SOIKE,* AMBHAN D. FELSENFELD, AND PETER J. GERONE

Department of Microbiology, Delta Regional Primate Research Center, Tulane University, Covington, Louisiana 70433

Received 26 November 1980/Accepted 22 March 1981

Replication of simian varicella virus (SVV) in Vero cell cultures was inhibited by acyclovir, 9-(2-hydroxyethoxymethyl)guanine (ACV), at a concentration of 10 $\mu\text{g}/\text{ml}$ in culture medium. Intravenous administration of ACV at 10 mg/kg twice a day for 10 days or 15 mg/kg three times a day for 5 days to patas monkeys (*Erythrocebus patas*) beginning 48 h after SVV inoculation blocked the appearance of rash and other clinical symptoms but did not affect viremia. ACV treatment of African green monkeys (*Cercopithecus aethiops*) at 10 mg/kg twice a day by intravenous injection beginning 24 or 72 h after SVV inoculation and continuing for 10 days had no effect on clinical symptoms, including the development of rash, or on the appearance of viremia. The minimal therapeutic results could be due to the observation that doses of 10 or 15 mg/kg produced plasma levels of ACV which were lower than 5 $\mu\text{g}/\text{ml}$, the concentration that inhibited SVV multiplication in vitro, and decayed rapidly.

The susceptibility of members of the herpesvirus group to inhibition in vitro by acyclovir [ACV; 9-(2-hydroxyethoxymethyl) guanine] has been reported to vary from the highly susceptible herpes simplex virus (HSV) type 1 to the markedly resistant cytomegalovirus (4). Clinical isolates of varicella-zoster virus (VZV) were shown to be inhibited by a mean 50% infectious dose of 3.75 μM for ACV, compared with 0.15 μM for HSV type 1 and 1.02 μM for HSV type 2. The greater resistance of VZV to ACV was confirmed by Biron and Elion, who reported a mean 50% infectious dose of 3.65 μM (1). This level of inhibition in vitro is clinically attainable and suggests potentially effective therapeutic treatment of human varicella infection (1, 4).

Experimentally induced HSV infections of various tissues in vivo have been effectively treated with ACV. These have included keratitis in rabbits (12), lip and skin lesions in mice (13, 15), and encephalitis (19) and ganglionic infections in mice (10, 13, 14, 17). Reports of ACV treatment of human herpesvirus infections are limited. Application of a 3% ACV ointment five times daily to herpesvirus corneal ulcers prevented recurrences of lesions that were observed to occur in patients receiving placebo (11). Intravenously (i.v.) administered doses of up to 5.0 mg/kg three times a day (t.i.d.) for 5 days were given to 23 patients with malignant cancers and

cutaneous or systemic HSV or VZV infections (20). Treatment appeared to arrest the infections, as evidenced by relief of pain and restricted dissemination and development of lesions. This study was uncontrolled, however, and requires further confirmation, since the course of the disease, even in immunosuppressed patients, can be self-limited. The dose of 15 mg/kg per day was without apparent toxicity. Pharmacokinetic studies have been conducted in 14 human patients who received a single i.v. dose of ACV varying from 0.5 to 5.0 mg/kg without exhibiting adverse effects (6). Multiple i.v. doses of 2.5, 5.0, or 10.0 mg/kg every 8 h for a total of three treatments were also nontoxic.

We have evaluated the antiviral activity of parenterally administered ACV in monkeys experimentally infected with simian varicella virus (SVV). Although an effect on clinical disease was observed in patas monkeys (*Erythrocebus patas*), viremia was not inhibited. African green monkeys (*Cercopithecus aethiops*), although responding similarly to SVV infection, did not show the same response to ACV treatment in either expression of clinical disease or development of viremia. Assays of plasma levels of ACV were performed in an attempt to explain the marginal therapeutic effects.

MATERIALS AND METHODS

Drug. ACV was provided by the Burroughs Wellcome Co., Research Triangle Park, N. C., in cooperation with the Antiviral Substances Program of the

† Publication 46 of the Cooperative Antiviral Testing Group.

National Institute of Allergy and Infectious Diseases. The drug was supplied as the sodium salt of ACV, dispensed as 500 mg of sterile powder in 100-ml vials. At the time of use, ACV was dissolved in sterile saline. The pH of the ACV solution was 10.3. Efforts to lower the pH to neutrality with 1 N HCl resulted in precipitation of the drug. No other acid or buffer solutions were used in attempts to reduce the pH. The drug was administered at the alkaline pH by slow i.v. infusion at a dose of 10 or 15 mg/kg over a period of 5 min or longer.

Virus. SVV was isolated from monkeys infected during a natural outbreak at this center. The virus was propagated and assayed for plaque-forming units (PFU) in Vero cell cultures in Eagle minimal essential medium with 10% fetal calf serum as previously described (9). The virus inoculum was prepared as a stock in 35% sorbitol at the fifth passage in Vero cells originating from infected monkey tissues. The pool was stored at -70°C and had a titer of 5×10^5 PFU/ml. When monkeys were experimentally infected with SVV, a titration was performed on the inoculum by inoculating logarithmic dilutions into Vero cell cultures.

HSV type 1 (strain F) and type 2 (strain G) were obtained from the American Type Culture Collection. The viruses were propagated in Vero cells, frozen and thawed one time, and centrifuged at 2,000 rpm for 20 min. The stock viruses were dispensed in 1.0-ml volumes and stored at -70°C . VZV strain Ellen, also obtained from the American Type Culture Collection, was grown in human lung fibroblast cells. The infected cells were scraped from the surface of the flasks, pooled, and frozen in 1.0-ml volumes in 35% sorbitol at -70°C .

In vitro studies. Replicate cultures of confluent Vero cells grown in 60-mm petri dishes were inoculated with HSV type 1, HSV type 2, or SVV at dilutions of the stock viruses which contained 100 to 200 PFU per culture. VZV was inoculated at a similar concentration into replicate petri dish cultures containing confluent monolayers of human lung fibroblasts. The growth medium for all cultures was Eagle minimal essential medium with 10% fetal calf serum. After 4 h of incubation at 37°C in 5% CO_2 in air to allow for virus adsorption, the unadsorbed virus was aspirated and the cultures were washed once. Fresh growth medium was added which contained varying concentrations of ACV. Concentrations of ACV of 50, 25, 10, 5, 1, 0.5, 0.25, and 0.1 $\mu\text{g}/\text{ml}$ were tested in triplicate against each virus. ACV was dissolved in Eagle minimal essential medium with 10% fetal calf serum for SVV and VZV; a similar medium containing 1% methylcellulose was used as an overlay medium of HSV types 1 and 2.

After 5 days of incubation, the cultures were examined for cytopathic changes, fixed with methanol, and stained with methylene blue-basic fuchsin; the number of plaques in each dish was then counted. The concentration of ACV which inhibited 80% or more of the plaques of each virus when compared with the plaques produced in the untreated control cultures was determined to be the endpoint and was termed the minimal inhibitory dose.

Monkeys. Monkeys were purchased for this study

as wild-caught feral animals from a commercial supplier. Sixteen African green monkeys (*C. aethiops*) from Kenya and 24 patas monkeys (*E. patas*) from Nigeria were used. After arrival at this center, the monkeys were quarantined for at least 90 days. Before use in antiviral studies, the monkeys were moved to an infection control unit of the center and held in individual cages. At this time, blood was drawn on two occasions to establish base-line hematology and clinical chemistry data and to conduct antibody assays to SVV.

The 16 African green monkeys were all female subadults ranging in weight from 2.0 to 3.2 kg. The 24 patas monkeys consisted of 18 females and 6 males; they were young adults weighing 3.0 to 6.3 kg, with most weighing 3.0 to 4.8 kg.

Plasma levels of ACV. Experiments were performed to determine concentrations of ACV in the plasma of monkeys after i.v. administration. In an initial experiment, four patas monkeys, weighing 3.6 to 4.8 kg, received ACV at 10 mg/kg. This dose was dissolved in 200 ml of saline and given as an i.v. drip over 30 min. At 0.5, 1, 2, 4, and 6 h after administration, blood was drawn in heparin and the plasma was separated for ACV analysis. A second group of four African green monkeys weighing 2.8 to 4.6 kg was given ACV at a dose of 10 mg/kg in a 5-min i.v. infusion. Plasma was collected on the same schedule as that followed for the patas monkeys and used for ACV assay of plasma levels.

In a final experiment, seven patas monkeys weighing 3.5 to 6.3 kg received ACV at a dose of 15 mg/kg in a 5-min i.v. infusion. Plasma was collected at 1 and 6 h after injection. Assays for ACV levels in plasma were performed by Paulo de Miranda, The Wellcome Research Laboratories, by radioimmunoassay (18).

Antiviral studies in monkeys. Monkeys determined to be free of SVV antibody were challenged with 10^5 PFU of the test virus. The inoculation procedure and the assessment of infection have been previously described (8, 22). In brief, 1.5 ml of a suspension of SVV was inoculated both intratracheally through the cricoid cartilage and i.v. The dual routes of inoculation with a total virus dose of 10^5 PFU per monkey produce consistently generalized infection in both patas and African green monkeys (A. D. Felsenfeld and K. F. Soike, unpublished data).

Two experiments were performed with patas monkeys to evaluate the antiviral activity of ACV on SVV infection. In the first experiment, 12 monkeys (5 males and 7 females) weighing 3.0 to 5.8 kg were divided into 3 groups of 4 monkeys. ACV treatment was begun 48 h after virus inoculation and was repeated twice daily, at 8 a.m. and 3 p.m. for 10 days. Four uninfected drug control monkeys and four SVV-infected monkeys were treated by dissolving the sodium salt of ACV in 200 ml of saline, which was administered at a dose of 10 mg/kg as an i.v. drip over 30 to 60 min with the monkeys restrained in primate chairs. Four untreated infection control monkeys received 200 ml of saline in the same manner. Plasma collected from the four drug control monkeys at 0.5, 1, 2, 4, and 6 h after ACV administration was assayed for ACV plasma levels. Two treatments per day, 6 to 7 h apart, were given, for a total

daily dose of 20 mg/kg. In a second experiment, eight patas monkeys were infected with SVV and two additional monkeys served as uninfected drug controls. The infected monkeys included eight females weighing 3.0 to 4.8 kg; one male (4.7 kg) and one female (6.3 kg) were used as drug controls. The sodium salt of ACV was dissolved in saline at 3 mg/ml and given at 5 ml/kg of body weight at a dose of 15 mg/kg. The drug was administered i.v. slowly over a 5-min period to monkeys sedated with Ketaset at 10 mg/kg. Three treatments per day were given, at 8 a.m., 2 p.m., and 8 p.m., for a total dose of 45 mg/kg per day. ACV treatment began 48 h after virus inoculation and was continued for 5 days. Plasma collected 1 and 6 h after ACV injection was assayed for ACV levels by P. de Miranda.

In a third experiment, 11 female African green monkeys weighing 2.0 to 3.2 kg were infected with SVV. Three monkeys received i.v. injections of saline at 5 ml/kg of body weight and served as infection controls. ACV treatment was begun in four monkeys 24 h after virus inoculation and in four additional monkeys 72 h after inoculation. ACV as the sodium salt dissolved in saline at 2 mg/ml, was administered in 10-mg/kg doses at 8 a.m. and 3 p.m. for 11 days.

Blood drawn on days 0, 3, 5, 7, and 9 after virus inoculation was used for hematology, clinical chemistry, and virus isolation. Serum was collected at 0, 14, and 21 days for determination of serum neutralizing antibody. Hematological evaluations included a complete blood count and differential. Biochemical parameters determined were blood urea nitrogen, serum creatinine, bilirubin, albumin, globulin, and serum transaminase. For virus isolation, lymphocytes were collected from 3.0 ml of heparinized blood by Ficoll-Hypaque gradient separation, washed twice in RPMI 1640 medium with 20% fetal calf serum, and inoculated into triplicate tubes of confluent Vero cell cultures (experiment 1) or in duplicate 25-cm² culture flasks of Vero cells (experiments 2 and 3). The cultures were maintained at 37°C in 5% CO₂ and air and observed microscopically for development of plaques. The infected flask cultures were stained with methylene blue-basic fuchsin stain, and the plaques were counted.

RESULTS

In vitro antiviral activity. The concentration of ACV required to inhibit 80% of SVV plaques was 10 µg/ml in the tissue culture medium. With the same 80% inhibition endpoint, 0.1 µg of ACV per ml was required for HSV type 1, 1.0 µg/ml was required for HSV type 2, and 2.5 µg/ml was required for VZV. These results indicate that SVV was susceptible to inhibition by ACV *in vitro* but was more resistant than HSV type 1 or 2 and showed an equivocal difference in susceptibility from human VZV.

In vivo studies of antiviral activity of ACV. Table 1 shows the effects of a daily ACV dose of 20 or 45 mg/kg on SVV infections in patas monkeys. In the initial experiment, the dose was titrated at 1.35×10^5 PFU per monkey. Treatment at 20 mg/kg, started 48 h after inoc-

ulation, did not affect the viremia. Virus was isolated from the lymphocytes of all untreated control and all four ACV-treated monkeys on days 5 and 7 after injection. However, ACV treatment did inhibit the development of clinical disease, expressed principally as a rash. Vesicular lesions, typical of the disease, appeared on the face, abdomen, and extremities of each infection control monkey by 9 days post-inoculation but were not observed in any of the four ACV-treated monkeys. One monkey (no. 7201) in the infected group receiving ACV died on day 12 of an unrelated bacterial pneumonia. No abnormalities in hematology or clinical chemistry values were encountered during the course of infection or treatment. Minor local irritation at the site of drug injection was occasionally noted. The required frequent i.v. administration with some infiltration of the drug at the site of injection resulted in occasional collapse and local damage to the vessels. Antibody to SVV was detected in 21 days after virus inoculation in the sera of all untreated control and ACV-treated monkeys.

In a second group of patas monkeys, each inoculated with 8.1×10^4 PFU, ACV was given at a daily dose of 45 mg/kg (15 mg/kg t.i.d.) for 5 days. No inhibition of viremia occurred, but there was an effect on the clinical disease. The mean number of plaques counted in two flasks inoculated with lymphocytes from each blood specimen from each monkey showed no appreciable quantitative differences (Table 1). Monkeys treated with ACV failed to develop the varicella rash noted in three of the four infected controls. Administration of ACV at a daily dosage 45 mg/kg produced no clinical signs of toxicity and was without effect on hematological or biochemical values. Antibody to SVV appeared in both groups of infected monkeys at similar titers.

Eleven African green monkeys were infected with SVV at a dose of 9.4×10^4 PFU. Three monkeys serving as untreated controls received saline twice daily i.v. ACV was administered i.v. at a daily dose of 20 mg/kg (10 mg/kg b.i.d.) to four monkeys starting 24 h after inoculation and continuing for 11 days. An additional four monkeys received similar ACV treatment commencing 72 h after virus injection and continuing for 9 days (Table 2). Viremia was detected in only two of the three control monkeys, although all three developed a rash. Viremia also occurred in each ACV-treated monkey regardless of the time treatment was initiated. The numbers of plaques developing in duplicate cultures of Vero cells were similar in the two treatment groups. Three of the four monkeys starting treatment at 24 h

TABLE 1. *Virus isolation and clinical disease in patas monkeys infected with SVV and treated i.v. with ACV*

Expt no.	Treatment group	Monkey no.	Viremia on day ^a :				Rash (day)	Antibody titer
			3	5	7	9		
1	Uninfected drug control ^b	7190	—	—	—	—	None	<1:8
		7198	—	—	—	—	None	<1:8
		7200	—	—	—	—	None	<1:8
		7203	—	—	—	—	None	<1:8
	Infection control	5787	+	+	+	—	+	1:512
		7195	—	+	+	—	+	1:512
		7207	—	+	+	—	+	1:512
		7208	—	+	+	—	+	1:512
	Infected, ACV treated ^b	7201	—	+	+	—	None	Died
		7202	—	+	+	—	None	1:256
		7204	—	+	+	—	None	1:256
		7205	—	+	+	—	None	1:64
2	Uninfected, drug control ^c	9202	—	—	—	—	None	<1:10
		9203	—	—	—	—	None	<1:10
	Infection control	9204	0.5	26	185	706	9-13	>1:640
		9205	0	8	142	85.5	10-11	>1:640
		9206	1	43.5	123	445	None	>1:640
		9207	0	8	68	52	10-13	1:640
	Infected, ACV treated ^c	9208	0	4	19.5	182.5	None	1:640
		9209	0	5	81	63	None	1:640
		9210	2.5	3.5	83.5	190	None	1:640
		9211	1.5	2.5	17	21	None	1:640

^a Mean number of plaques developing in paired flasks. +, Plaques present.^b ACV at 20 mg/kg per day b.i.d. beginning 48 h after virus inoculation.^c ACV at 45 mg/kg per day t.i.d. beginning 48 h after virus inoculation.TABLE 2. *Virus isolation and clinical disease in African green monkeys infected with SVV and treated i.v. with ACV*

Treatment group	Monkey no.	Viremia on day ^a :				Rash (day)	Antibody titer
		3	5	7	9		
Infection control	8178	0	0	0	0	7-11	<1:8
	8179	0	0	7	0	7-11	1:128
	8325	0	61	83	0	7-11	>1:512
Infected, ACV treated ^b 24 h post-infection	8174	0	50.5	5	0	10-11	>1:512
	8177	+	31.5	98.5	0	9-11	1:512
	8182	0	11	153	0	9-11	>1:512
	8326	3	20	0	0	None	>1:512
	8175	3	16.5	14	0	7-11	1:256
72 h post-infection	8180	3.5	5.5	368	0	8-11	1:128
	8323	2	23.5	87	0	7-11	1:256
	8324	+	190	>1,000	0	11-14	1:512

^a See Table 1.^b ACV at 20 mg/kg/day b.i.d.

developed a rash that was slightly delayed in appearance compared with the time of appearance in the infection control groups. All four of the monkeys in which treatment was begun at 72 h developed rash. Antibody to SVV appeared in 10 of the 11 monkeys. The one exception was infection control monkey 8178, which developed a rash but no viremia. Sera from this monkey examined for antibody at 4 and 6 weeks after

virus inoculation also were without antibody. Reinoculation of monkey 8178 with SVV at 6 weeks with 6.3×10^4 PFU of virus did not result in viremia or rash, although antibody to SVV was demonstrated at a titer of 1:128 in serum examined at 3 weeks after the second injection. Hematology and clinical chemistry data from the African green monkeys remained within normal limits with the exception of the serum trans-

aminase levels (serum glutamic oxalacetic transaminase [SGOT] and serum glutamic pyruvic transaminase [SGPT]). The transaminase values, which are usually observed to rise in SVV infection, reflecting systemic involvement, increased in both control and ACV-treated monkeys. The increase in both SGOT and SGPT occurred 3 days after virus injection and persisted through day 9. By day 14, as the clinical disease began to subside, the values declined to normal limits.

Plasma levels of ACV. Table 3 reports the results of radioimmunoassays performed by P. de Miranda of Burroughs Wellcome Laboratories on sera from monkeys receiving ACV. Plasma levels of ACV were determined in relation to time after i.v. administration. In both patas and African green monkeys which received an injection of 10 mg/kg, the maximal plasma levels of ACV were achieved early, within 30 min of the time of administration. The levels of ACV at 30 min reached 3.66 $\mu\text{g/ml}$ in patas monkeys and 2.65 $\mu\text{g/ml}$ in African green monkeys. The plasma concentration of ACV declined rapidly, exhibiting a half-life of about 1 h. At 6 h, ACV concentrations were still detectable but extremely low. The plasma levels of ACV attained after injection of 10 mg/kg were less than the 5 $\mu\text{g/ml}$ required for 80% inhibition of SVV in vitro. Increasing the dose of ACV to 15 mg/kg in patas monkeys did result in higher plasma concentrations, but at 1 h after drug administration, the plasma level was below that required for therapeutic efficacy based on the in vitro data.

Clinical observations. There was no evidence of toxicity in patas monkeys receiving daily ACV doses of 45 mg/kg for 5 days. Hema-

tology and clinical chemistry values remained within normal limits. Transaminase levels, which normally rise in untreated SVV infection, also rose in the ACV-treated monkeys (Table 4). The data suggest that recovery was hastened by ACV treatment, since the values returned to normal earlier in the treated monkeys. Transaminase values on day 9 in the control group and on day 5 in the ACV-treated group were notably high because of high levels present in one monkey in each group on those days, as reflected in the large standard errors. Values for blood urea nitrogen determinations also showed spotty increases for both untreated and ACV-treated monkeys.

Treatment at 20 mg/kg per day similarly was without apparent toxicity. Again, transaminases were the only biochemical parameter that increased. The higher values for SGOT and SGPT occurred in both control and ACV-treated monkeys and returned to normal levels in both groups as the clinical disease subsided.

DISCUSSION

Differences in susceptibility to ACV among the major viruses within the herpes group have been reported (1, 4). Even among strains of HSV, differences in susceptibility to ACV have been observed (4, 5). Strains of HSV with increased resistance to ACV have been shown to emerge after exposure to the drug (3, 19, 21). Human VZV as well as SVV were found to be more resistant to inhibition in vitro by ACV than were HSV type 1 or type 2. As the mode of action of ACV is through virus-induced thymidine kinase, differences occurring in the induction of the enzyme by the various viruses are presumably responsible for these variations in inhibition. Human varicella virus does induce a virus-specific thymidine kinase (1, 2, 7). SVV has been shown to code for a specific thymidine kinase which phosphorylates ACV (K. K. Biron, J. A. Fyfe, and G. B. Elion, personal communication).

In this study, ACV was shown to have a minimal effect on the course of SVV infection in patas monkeys. Treatment did not affect viremia and virus replication but did modify clinical expressions of disease. Doses of 20 and 45 mg/kg per day inhibited development of the rash usually associated with the disease and improved the health of the monkeys whose activity and appetite remained normal whereas untreated control animals exhibited lethargy and anorexia. ACV administration did not affect the antibody response to SVV, since control and treated monkeys developed substantial antibody titers in response to active viral replication in

TABLE 3. Plasma levels of ACV in patas and African green monkeys after i.v. administration of the monosodium salt of ACV

Mon-key species	No. of monkeys	ACV dose (mg/kg)	Time after administration (h)	Mean plasma concn ($\mu\text{g/ml}$ $\pm$ standard error)
Patas	4	10	0.5	3.66 $\pm$ 0.25
			1	1.51 $\pm$ 0.20
			2	0.75 $\pm$ 0.02
			4	0.26 $\pm$ 0.02
			6	0.12 $\pm$ 0.02
Patas	7	15	1	3.08 $\pm$ 0.22
			6	0.54 $\pm$ 0.16
African green	4	10	0.5	2.65 $\pm$ 0.49
			1	1.31 $\pm$ 0.05
			2	0.71 $\pm$ 0.09
			4	0.27 $\pm$ 0.07
			6	0.12 $\pm$ 0.02

TABLE 4. Mean transaminase values (SGOT and SGPT) and blood urea nitrogen levels in untreated control and ACV-treated patas monkeys infected with SVV

Group	Days post-infection	SGOT (IU/ml)	SGPT (IU/ml)	Blood urea nitrogen (mg/100 ml)
Control	-3	70.00 $\pm$ 7.68	40.50 $\pm$ 5.23	24.87 $\pm$ 2.07
	3	160.50 $\pm$ 28.84	84.75 $\pm$ 13.21	34.13 $\pm$ 3.22
	5	172.00 $\pm$ 35.76	100.75 $\pm$ 8.18	39.15 $\pm$ 4.49
	7	236.75 $\pm$ 35.01	128.50 $\pm$ 20.30	32.52 $\pm$ 3.33
	9	513.50 $\pm$ 257.78	494.50 $\pm$ 269.24	27.85 $\pm$ 3.43
	13	160.00 $\pm$ 32.16	157.25 $\pm$ 39.03	
	18	149.50 $\pm$ 65.30	112.75 $\pm$ 56.84	
ACV ^a	-3	52.25 $\pm$ 4.24	40.00 $\pm$ 2.88	21.12 $\pm$ 1.16
	3	165.25 $\pm$ 25.99	87.50 $\pm$ 4.97	33.65 $\pm$ 2.99
	5	545.00 $\pm$ 335.78	344.25 $\pm$ 231.37	27.12 $\pm$ 1.04
	7	279.50 $\pm$ 76.92	366.25 $\pm$ 241.36	29.60 $\pm$ 1.64
	9	96.50 $\pm$ 15.91	258.25 $\pm$ 159.35	29.52 $\pm$ 1.65
	13	88.50 $\pm$ 13.66	102.50 $\pm$ 18.16	
	18	61.25 $\pm$ 6.20	54.50 $\pm$ 6.19	

^a Dose of 45 mg/kg per day on days 2 through 6.

both groups.

These results of ACV treatment are consistent with those reported by Selby et al. (20) in immunosuppressed human patients with diagnosed VZV infection. ACV treatment of those patients brought relief from pain, regression of skin lesions and inhibition of development of new skin lesions. Although no virological parameters were reported in the human study that allowed comparison with the SVV monkey model, both studies suggest clinical effectiveness.

Similar treatment of SVV-infected African green monkeys with ACV at 20 mg/kg per day was without appreciable beneficial effects. SVV viremia was not inhibited by ACV treatment, and the varicella-like rash was observed to develop in treated animals. A slight moderation in the time of appearance of the rash may have occurred in those monkeys in which treatment began 24 h after virus inoculation.

The failure of ACV to inhibit the replication of SVV may have reflected inadequate plasma levels of ACV occurring after i.v. administration and a relatively rapid disappearance of ACV from the plasma. The pharmacokinetics of ACV in monkeys do differ from those in humans (6). With doses of 10 mg/kg, plasma concentrations were 3.66 μ g/ml in patas monkeys and 2.65 μ g/ml in African green monkeys. In humans, ACV at 5 mg/kg produced plasma levels of 7.6 μ g/ml. The half-life of ACV was 2.1 to 3.8 h in human subjects and approximately 1 h in monkeys. Although higher plasma levels were produced by 15-mg/kg doses of ACV the concentration at 1 h was lower than the 5- μ g/ml level shown to be required for inhibition of SVV in vitro. It is possible that higher doses produce plasma levels inhibitory to the virus. The possibility was con-

sidered that the subcutaneous route of administration might provide a more sustained plasma level of ACV. Accordingly the drug was administered at a dose of 15 mg/kg to one patas monkey. Three injections in different but adjacent sites on the abdomen and chest were given daily for 5 days. Within 24 h each injection site became erythematous, vesiculated, and extensively inflamed. Sloughing eventually occurred. On the 3rd day, serum transaminase levels rose to abnormal values which returned to normal within 3 days of the last injection. These results precluded the use of the subcutaneous route for treatment.

The possibility of administering ACV only was also explored. ACV was dissolved in the drinking water at 1 mg/ml and provided ad libitum to three African green monkeys over a 6-h period. At the end of this time, the consumed dose was calculated and plasma was assayed for ACV concentrations. Doses of 49, 41, and 32 mg/kg were absorbed in the 6-h period which resulted in plasma levels of 0.71, 1.26, and 0.80 μ g/ml, respectively. These plasma levels would presumably be too low for effective therapeutic results based on values obtained in in vitro susceptibility studies with SVV.

ACKNOWLEDGMENTS

We thank Mary Barbee and Stephen Williams for technical assistance; Ann Quiroz for typing and editing of the manuscript; P. de Miranda, Burroughs Wellcome Research Laboratories, Research Triangle Park, N. C., for performing the radioimmunoassays for ACV; G. B. Elion, Burroughs Wellcome Research Laboratories, for constructive comments in reviewing the manuscript; and K. K. Biron and J. A. Fyfe for confirming the existence of a thymidine kinase directed by SVV.

This work was supported under Public Health Service contract AI 52525 from the Antiviral Substances Program of the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Biron, K. K., and G. B. Elion. 1980. In vitro susceptibility of varicella-zoster virus to acyclovir. *Antimicrob. Agents Chemother.* 18:443-447.
2. Cheng, Y.-C., T. Y. Tsou, T. Hackstadt, and L. P. Mallovia. 1979. Induction of thymidine kinase and DNase in varicella-zoster virus-infected cells and kinetic properties of the virus-induced thymidine kinase. *J. Virol.* 31:172-177.
3. Coen, D. M., and P. A. Schaffer. 1980. Two distant loci confer resistance to acycloguanosine in herpes simplex virus type 1. *Proc. Natl. Acad. Sci. U. S. A.* 77:2265-2269.
4. Crumpacker, C. S., L. E. Schnipper, J. A. Zaia, and M. J. Levin. 1979. Growth inhibition by acycloguanosine of herpesvirus isolated from human infections. *Antimicrob. Agents Chemother.* 15:642-645.
5. DeClerq, E., J. Descamps, G. Verhelst, R. T. Walker, A. S. Jones, P. F. Torrence, and D. Shugar. 1980. Comparative efficacy of anti-herpes drugs against strains of herpes simplex virus. *J. Infect. Dis.* 141:563-574.
6. DeMiranda, P., R. J. Whitely, M. R. Blum, R. E. Keeny, N. Barton, D. M. Cocchetto, S. Good, G. P. Hemstreet, L. E. Kirk, D. A. Page, and G. B. Elion. 1979. Acyclovir kinetics after intravenous infusion. *Clin. Pharmacol. Ther.* 26:718-728.
7. Doberson, M. J., M. Jerkofsky, and S. Greer. 1976. Enzymatic basis for the selective inhibition of varicella-zoster virus by 5-halogenated analogs of deoxycytidine. *J. Virol.* 20:478-486.
8. Felsenfeld, A. D., C. R. Abee, P. J. Gerone, K. F. Soike, and S. R. Williams. 1978. Phosphonoacetic acid in the treatment of simian varicella. *Antimicrob. Agents Chemother.* 14:331-335.
9. Felsenfeld, A. D., and N. J. Schmidt. 1975. Immunological relationship between Delta herpesvirus of patas monkeys and varicella-zoster virus of humans. *Infect. Immun.* 15:807-812.
10. Field, H. J., S. E. Bell, G. B. Elion, A. A. Nash, and P. Wildy. 1979. Effect of acycloguanosine treatment on acute latent herpes simplex infections in mice. *Antimicrob. Agents Chemother.* 15:554-561.
11. Jones, B. R., D. J. Coster, P. N. Fison, G. M. Thompson, L. M. Cobo, and M. G. Falcon. 1979. Efficacy of acycloguanosine (Wellcome 248U) against herpes simplex corneal ulcers. *Lancet* i:243-244.
12. Kaufman, H. E., E. D. Vernell, Y. M. Centifanto, and S. D. Rheinstrom. 1978. Effect of 9-(2-hydroxyethoxymethyl) guanine on herpesvirus-induced keratitis and iritis in rabbits. *Antimicrob. Agents Chemother.* 14:842-845.
13. Klein, R. J., A. E. Friedman-Kien, and E. De Stefano. 1979. Latent herpes simplex virus infections in sensory ganglia of hairless mice prevented by acycloguanosine. *Antimicrob. Agents Chemother.* 15:723-729.
14. Mayo, D. R., J. C. Richards, and F. Rapp. 1979. Acycloguanosine treatment of herpesvirus infections in footpads and nervous tissue of normal and immunosuppressed mice. *Intervirology* 12:345-348.
15. Park, N. H., P. Pavan-Langston, M. Q. Hettinger, S. L. McLean, D. M. Albert, T. S. Lin, and W. H. Prusoff. 1980. Topical therapeutic efficacy of 9-(2-hydroxyethoxymethyl) guanine and 5-iodo-5' dideoxyuridine on oral infection with herpes simplex virus in mice. *J. Infect. Dis.* 141:575-579.
16. Park, N. H., D. Pavan-Langston, and S. L. McLean. 1979. Acyclovir in oral and ganglionic herpes simplex virus infections. *J. Infect. Dis.* 140:802-806.
17. Park, N. H., D. Pavan-Langston, S. L. McLean, and D. N. Albert. 1979. Therapy of experimental herpes simplex encephalitis with acyclovir in mice. *Antimicrob. Agents Chemother.* 15:775-779.
18. Quinn, R. P., P. de Miranda, L. Gerald, and S. S. Good. 1979. A sensitive radioimmunoassay for the antiviral agent BW248U (9-(2-hydroxyethoxymethyl) guanine). *Anal. Biochem.* 98:319-328.
19. Schnipper, L. E., and C. S. Crumpacker. 1980. Resistance of herpes simplex virus to acycloguanosine: role of viral thymidine kinase and DNA polymerase loci. *Proc. Natl. Acad. Sci. U. S. A.* 77:2270-2273.
20. Selby, P. J., et al. 1979. Parenteral acyclovir therapy for herpesvirus infections in man. *Lancet* ii:1267-1270.
21. Smith, K. O., W. L. Kennell, R. H. Poirier, and F. T. Lynd. 1980. In vitro and in vivo resistance of herpes simplex virus to 9-(2-hydroxyethoxymethyl) guanine (acycloguanosine). *Antimicrob. Agents Chemother.* 17:144-150.
22. Soike, K. F., A. D. Felsenfeld, S. Gibson, and P. J. Gerone. 1980. Ineffectiveness of adenine arabinoside and adenine arabinoside 5'-monophosphate in simian varicella infection. *Antimicrob. Agents Chemother.* 18:142-147.