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Antiherpesvirus Activity of 9-(4-Hydroxy-3-Hydroxymethylbut-1-yl) Guanine (BRL 39123) in Animals

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The antiviral activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL 39123) was assessed in several animal models of herpes simplex virus (HSV) infection. BRL 39123 was as active as acyclovir (ACV) when applied topically to guinea pigs with a cutaneous HSV type 1 (HSV-1) infection and was also active topically in an HSV-2 genital infection. Before systemic administration to infected animals, BRL 39123 and ACV were administered orally and subcutaneously to mice, and the blood was assayed for each compound by high-pressure liquid chromatography. When given systemically to mice infected cutaneously with HSV-1, BRL 39123 was as active as ACV. In mice infected intranasally with HSV-1 or HSV-2, single daily subcutaneous doses of BRL 39123 were more effective than equivalent treatment with ACV, reflecting the more persistent activity seen in cell culture and a more stable triphosphate within the infected cell. When the compounds were supplied in drinking water for this infection, BRL 39123 and ACV had similar potencies against HSV-1, although ACV was more active against an HSV-2 infection than BRL 39123 was. In mice infected intraperitoneally with HSV-1, BRL 39123 was 10-fold more potent than ACV and a single dose of BRL 39123 reduced virus replication within the peritoneal cavity more effectively than 3 doses of ACV given 1, 5, and 20 h after infection. Although BRL 39123 failed to eradicate the virus from mice latently infected with HSV-1, treatment initiated 5 h after infection of the ear pinna reduced the numbers of mice that developed latent infections.

The activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine (BRL 39123) against members of the herpesvirus family in cell culture has been described by Boyd et al. (4). In virus yield reduction assays, it was shown that BRL 39123 was more active than acyclovir (ACV) against herpes simplex virus type 1 (HSV-1) and of equal activity against HSV-2, although in plaque reduction assays BRL 39123 was less active than ACV against HSV-1 and HSV-2. It was demonstrated that treatment of HSV-infected MRC-5 cells with BRL 39123 for short periods inhibited virus replication much more effectively than similar treatment with ACV and that, in addition, virus replication remained depressed for prolonged periods after treatment with BRL 39123 (4). It was suggested that these results reflected different rates of phosphorylation for BRL 39123 and ACV and that the phosphorylated form of BRL 39123 was trapped efficiently within the infected cell.

Since BRL 39123 is a potent inhibitor of HSV in vitro, the antiviral activity of this compound was studied in several animal models of HSV infection. Moreover, since cell culture studies showed that the continuous presence of BRL 39123 in the extracellular medium was not necessary for the maintenance of viral inhibition, it was predicted that persistent activity would be observed in infected animals. In this report, results of experiments are described in which BRL 39123 was tested for efficacy in several HSV infections in animals and its activity was compared with that of ACV. The data extend the observations made independently by others (5, 14, 15, 19) describing the activity of BRL 39123 in animal models of HSV infections.

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MATERIALS AND METHODS

Animals. BALB/c and DBA/2 mice were obtained from Bantin and Kingman, Hull, United Kingdom, and hairless mice (Hr/Hr) and guinea pigs were obtained from Olac (1976), Bicester, United Kingdom. Group sizes were kept to the minimum required for statistical evaluation.

Viruses. HSV-1(SC16) (11) and HSV-2(10), a recent clinical isolate, were obtained from H. J. Field, University of Cambridge, Cambridge, United Kingdom. HSV-1(19312), a recent clinical isolate, was provided by H. Cummin, Public Health Laboratory, Epsom, United Kingdom, and HSV-2(MS) was obtained from the American Type Culture Collection, Rockville, Md. Stocks were prepared in BHK-21 cells.

Compounds. BRL 39123 and ACV were prepared as previously described within the laboratories of Beecham Pharmaceuticals (9, 16). For topical use, the compounds were prepared in aqueous cream (British Pharmacopoeia) and for other routes of administration, they were prepared as suspensions in 1% carboxymethyl cellulose (BDH, Poole, United Kingdom [British Drug Houses]) with 1% Tween 80 (Sigma Chemical Co., Poole, United Kingdom) in deionized water. For certain experiments, the compounds were dissolved in water and the mice were allowed to drink ad libitum.

Bioavailability in mice. At each time point (15, 60, and 180 min after drug administration), three mice were bled by cardiac puncture under CO_2 anesthesia. Equal volumes (0.2 ml) of blood from each mouse were pooled and treated with 16% trichloroacetic acid (0.6 ml; BDH), and after centrifugation to remove precipitated blood proteins, the supernatants were assayed by high-pressure liquid chromatography (HPLC).

Cutaneous infections. Male guinea pigs (200 to 300 g) were infected with HSV-1(19312) (3×10^4 PFU) by using a method similar to that of Alenius and Öberg (1), except that the animals were anesthetized with Hypnorm (Crown Chemicals, Lamberhurst, United Kingdom) and infected after scarification of six inoculation sites in the dorsal area with a 27-gauge needle. Groups of six animals were treated topically twice daily with BRL 39123 or ACV (1% [wt/wt] in aqueous cream) or vehicle alone from days 1 to 4. Approximately 50 μl per dose was applied to each inoculation site. The severity of the lesions was assessed daily as follows: 0, no lesions; 1, erythema or few isolated vesicles; 2, multiple small vesicles; 3, coalesced vesicles; and 4, crusted vesicles.

Male hairless mice (15 to 25 g) were anesthetized with diethyl ether and infected in the lumbar area with approximately 10^4 PFU of HSV-1(SC16) per site as previously described (10). Groups of 16 animals were treated subcutaneously or orally with BRL 39123 or ACV (50 mg/kg of body weight) or vehicle alone twice daily for 5 days, with treatment commencing at the time of infection. Lesions were assessed daily by using a revised scoring system: 0, no sign of infection; 1, vesicle at infection site; 2, scab at infection site; 3, distal lesions; 4, spreading lesion; 5, severe spreading lesion; and 6, some hind limb paralysis.

Female BALB/c mice were anesthetized by intraperitoneal injection of Hypnorm and Hypnovel (Roche Products, Welwyn Garden City, United Kingdom). The right ear pinna of each mouse was lightly scarified, and 10^5 PFU of HSV-1(SC16) in 20 μl of phosphate-buffered saline with 10% newborn calf serum was placed on the scarified area. The method, based on that of Hill et al. (11), consistently produced >95% infection, evidence of which was first seen 3 days after inoculation. Maximum severity was reached by about day 8, and by day 17 the lesions had resolved. The severity of the lesions was assessed daily as follows: 1, erythema; 2, isolated vesicles; and 3, coalesced vesicles. Groups of 30 animals were treated with BRL 39123 or ACV (100 mg/kg) or vehicle alone administered subcutaneously as a single daily dose for 5 days, commencing 5 h after infection. The right second, third, and fourth cervical ganglia were removed from mice 4 weeks after infection and examined for the presence of latent virus as described elsewhere (11).

Genital infection. Female guinea pigs (200 to 300 g) were inoculated intravaginally with HSV-2(MS) (3×10^3 PFU per animal) by using the method of Fraser-Smith et al. (7). The animals were examined daily and scored as follows: 0, no visible lesions; 1, erythema or one or two small lesions; 2, several small discrete lesions; 3, large discrete lesions; 4, coalesced lesions; and 5, ulcerating lesions. Animals were treated topically twice daily with either BRL 39123 (2% [wt/wt] in aqueous cream) or vehicle alone from days 0 to 10. Approximately 50 μl per dose was applied intravaginally and to the external genitalia. A total of 10 animals were treated with BRL 39123, and 10 were treated with vehicle alone.

Intranasal infection. Female BALB/c mice (12 to 14 g) were inoculated intranasally under light diethyl ether anesthesia with 50 μl of phosphate-buffered saline containing 10 50% lethal doses of HSV-1(SC16) (7×10^4 PFU) by using a method similar to that of Field et al. (6). Groups of 10 or 20 mice were treated with BRL 39123 or ACV administered either subcutaneously or in the drinking water. The dose levels and treatment times are shown in Table 2. The animals were observed for mortality for up to 21 days after infection.

Intraperitoneal infection. Female DBA/2 mice (12 to 14 g) were infected intraperitoneally with 5×10^5 PFU of HSV-1(SC16) in 0.1 ml of phosphate-buffered saline as described elsewhere (13). The mice were treated subcutaneously with BRL 39123, ACV, or vehicle alone. The dosages are described in Table 3 or in the text. At various times after inoculation, groups of five mice were killed and peritoneal washings were collected by injecting 2.0 ml of ice-cold minimal essential medium with 10% newborn calf serum into the peritoneal cavity. The abdomen of the mouse was gently massaged, and a sample of fluid was withdrawn. After three freeze-thaw cycles, the virus content of the samples was determined by plaque assay.

HPLC analysis. BRL 39123 and ACV concentrations were determined by HPLC (Waters Associates, Inc., Milford, Mass.). A Nova-Pak C_{18} cartridge (10 cm by 8 mm) fitted in a Z-module and protected with a C_{18} Guard-Pak was used in conjunction with two M45 pumps, a WISP autosampler, an M720 controller, an M730 data module, and a Spectroflow 757 detector (Kratos Analytical Instruments, Ramsey, N.J.). Two buffers were used; buffer A consisted of 50 mM sodium hydrogen phosphate in water, and buffer B consisted of 5 mM sodium dihydrogen phosphate in 80% methanol. Each sample was eluted at a flow rate of 1.6 ml/min by using a linear gradient changing from 1% buffer B and 99% buffer A at 1.5 min to 95% buffer B and 5% buffer A at 20 min. The column was equilibrated for 10 min before each sample injection. BRL 39123 and ACV had retention times of approximately 13.5 and 11.6 min, respectively, and were detected at 254 nm. The limits of detection were 0.2 and 0.25 μg/ml, respectively.

Statistical analysis. The analysis of variance was used for the guinea pig cutaneous infection and the mouse ear pinna infection. The Kolmogorov-Smirnov two-sample test was used for hairless mice, the Mann-Whitney test was used for the intraperitoneal infection in mice, and the Peto log rank test was used for the intranasal infection in mice. A value of P less than 0.05 was considered statistically significant.

RESULTS

Concentrations of BRL 39123 in blood. Concentrations of BRL 39123 in blood after oral and subcutaneous administration to BALB/c mice are shown in Table 1. To try to relate these concentrations with antiviral activity in a mouse cell line in vitro, the activities of BRL 39123 and ACV against HSV-1(SC16) and HSV-2(MS) in BALB/c 3T3 cells were determined. From plaque reduction assays (4), 50% inhibitory concentrations (IC_{50}s) of 0.07 and 0.02 μg/ml were obtained against HSV-1, and IC_{50}s of 0.11 and 0.03 μg/ml were obtained against HSV-2 for BRL 39123 and ACV, respectively.

Cutaneous infection and topical treatment. Figure 1 shows the effect of topical BRL 39123 or ACV treatment of guinea pigs cutaneously infected in the dorsal area with HSV-1(19312). Both compounds reduced the severity of the skin lesions caused by HSV-1 ($P < 0.001$). The mean time to healing was 11 days for both BRL 39123 and ACV, compared with >16 days for placebo-treated animals. There were no statistically significant differences between the two treatments when either the times to the maximum score or the areas under the curve were compared.

Cutaneous infection and systemic treatment. Hairless mice were infected on the flank with HSV-1(SC16) and treated with either BRL 39123 or ACV. Subcutaneous treatment with either BRL 39123 or ACV was effective against the cutaneous manifestation of the disease (Fig. 2a). The mean lesion scores were significantly different from those of con-

TABLE 1. Concentration of BRL 39123 and ACV in blood after single-dose oral and subcutaneous administration to BALB/c mice

Dose of BRL 39123 or ACV (mg/kg)	Concn (μg/ml) of BRL 39123 (ACV) at the indicated time					
	Oral administration			Subcutaneous administration		
	15 min	60 min	180 min	15 min	60 min	180 min
100	1.9	1.6	0.2	18	13	0.3
50	1.1	1.1	0.2	21	6.6	<0.2
50[a]	(8.5)	(4.3)	(<0.25)	(28)	(2.9)	(0.3)
25	0.5	0.6	<0.2	8.1	2.2	<0.2
12.5	0.4	0.3	<0.2	4.0	0.8	<0.2
6.25	0.3	<0.2	<0.2	1.8	<0.2	<0.2

[a] Dose of ACV.

trols for both compounds on days 6, 7, and 8 ($P < 0.001$). The activity after oral administration was less pronounced and reached statistical significance for both compounds only on day 6 after infection (Fig. 2b).

Inoculation of the ear pinna of BALB/c mice with HSV-1 results in an infection which is usually self-limiting and has a low mortality (8). The effect of subcutaneous BRL 39123 or ACV treatment on this infection is shown in Fig. 3. Mice were also treated with compound concentrations of 50 and 200 mg/kg (data not shown), and a dose-related response to each compound was seen. Treatment with either compound in this experiment was highly effective in reducing the lesion score ($P < 0.01$). Although there was a tendency at each dose level for lesions in mice treated with BRL 39123 to heal more rapidly than those in mice treated with ACV, the difference was not statistically significant.

Effect on HSV-1 latency. Mice from the experiment depicted in Fig. 3 were killed 28 days after infection, and the second, third, and fourth cervical ganglia were removed and tested for the presence of latent virus by in vitro culture. In the control group, virus was recovered from 18 of 20 mice (90%). In the high- and low-dose groups treated with BRL 39123, virus recovery was reduced to 63 and 83%, respectively, and to 48 and 67%, respectively, in the corresponding ACV groups. Thus, treatment with either compound initiated 5 h after infection caused a small decrease in the numbers of mice that developed latent infection.

A test was carried out to see whether treatment with BRL 39123 affected an established latent infection. Accordingly, 30 BALB/c mice infected with HSV-1(SC16) in the ear pinna 40 days previously, all of which had recovered from the primary infection, were treated orally for 14 days by incorporating the compound in the drinking water. The mean daily intake of BRL 39123 was estimated to be 100 mg/kg per day. Seven days after treatment was ceased, the cervical ganglia were removed and virus was subsequently isolated from all of the treated mice. Treatment of an established latent infection with BRL 39123 was thus unable to eradicate latent virus.

Genital infection. BRL 39123 was applied topically to the genital area of female guinea pigs infected intravaginally with HSV-2(MS). Since 0 of 10 treated animals developed severe lesions (lesion score, ≥3), compared with 4 of 10 animals in the vehicle group, BRL 39123 was clearly active.

Systemic infection. Intranasal inoculation of BALB/c mice with either HSV-1 or HSV-2 results in rapid entry of the virus into the central nervous system after initial replication in the respiratory epithelium, and the infection is almost invariably fatal (2, 6). BRL 39123 administered subcutaneously or orally was active against HSV-1 and HSV-2 in this model (Table 2). Furthermore, single daily subcutaneous doses of BRL 39123 were more effective than single daily doses of ACV. When administered in the drinking water, with administration commencing at the time of infection or 24 h after infection, the two compounds had similar activities against HSV-1, but ACV was more active than BRL 39123 against HSV-2 ($P < 0.01$).

HSV inoculated into the peritoneal cavity of DBA/2 mice replicates locally (13). By sampling the peritoneal contents and titrating infectious virus, growth curves can be constructed (Table 3). A single 50-mg/kg subcutaneous dose of BRL 39123 5 h after infection produced a greater reduction in peritoneal virus titers at 24 and 48 h than the same dose of

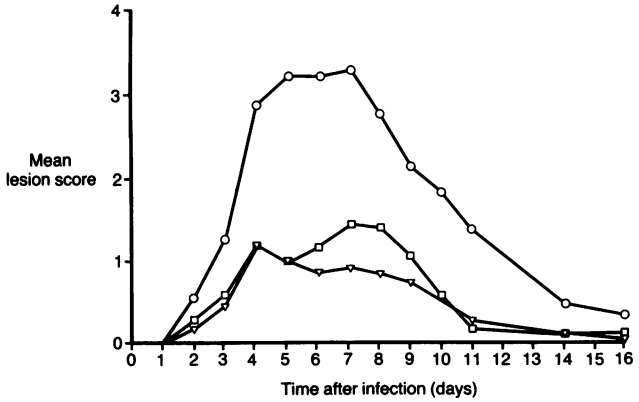

FIG. 1. Effect of topical BRL 39123 or ACV treatment in guinea pigs cutaneously infected on day 0 with HSV-1(19312). Groups of six animals were treated with vehicle (○), BRL 39123 (□), or ACV (▽), each prepared as a 1% suspension in aqueous cream and applied at the rate of 50 μl per inoculation site twice daily for 6 days, with treatment commencing 24 h after infection.

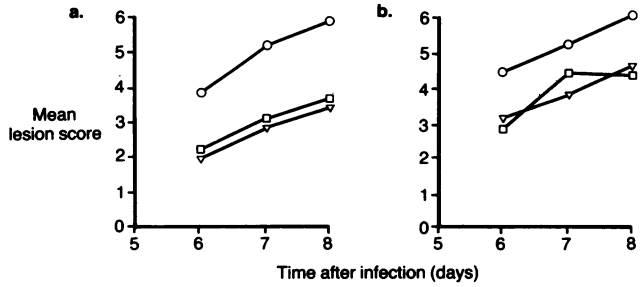

FIG. 2. Effect of subcutaneous (a) and oral (b) treatments with BRL 39123 or ACV in hairless mice cutaneously infected on day 0 with HSV-1(SC16). Groups of 16 mice were treated with vehicle alone (○), BRL 39123 (□), or ACV (▽), each administered at a concentration of 50 mg/kg per dose twice daily for 5 days, with treatment commencing at the time of infection.

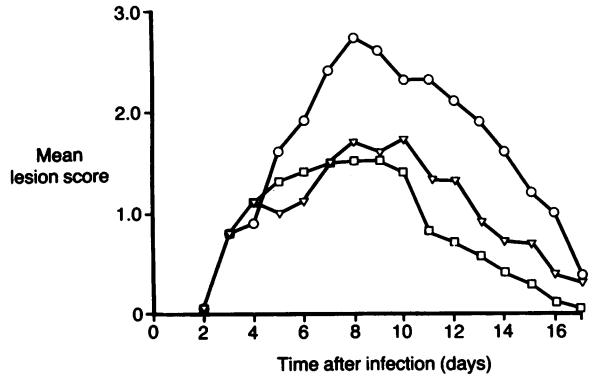

FIG. 3. Effect of subcutaneous BRL 39123 or ACV treatment (100 mg/kg per day) in BALB/c mice cutaneously infected in the ear pinna with HSV-1(SC16). Groups of 30 mice were treated with vehicle alone (○), BRL 39123 (□), or ACV (▽), each administered as a single daily subcutaneous dose for 5 days, commencing 5 h after infection.

ACV did ($P < 0.01$). Moreover, a single 50-mg/kg dose of BRL 39123 5 h after infection was more active than 3 50-mg/kg doses of ACV given 1, 5, and 20 h after infection ($P < 0.05$). It was also observed that a single 5.5-mg/kg dose of BRL 39123 given 24 h after infection reduced the amount of virus detected 48 h after infection more effectively than a single 50-mg/kg dose of ACV did ($P < 0.05$). Neither BRL 39123 nor ACV could be detected by HPLC in peritoneal wash samples at the time samples were taken for the virus assay. Since these samples were diluted at least 10-fold before the virus assay in Vero cells, it is unlikely that residual drug affected virus titers. BRL 39123 and ACV were detected in peritoneal washings (0.4 to 0.6 μg/ml and 0.6 to 1.8 μg/ml, respectively) during the first 30 min after administration of a 50-mg/kg dose, but these concentrations cannot account for the superior activity of BRL 39123 in this model, since the concentrations of ACV were higher than those of BRL 39123.

During these studies, in which BRL 39123 was administered to animals orally, subcutaneously, and topically to a maximum systemic daily dose of 250 mg/kg, no evidence of intolerance was observed. A toxicological evaluation of BRL 39123 is in progress and will be reported independently.

DISCUSSION

When prepared at 1% (wt/wt) in aqueous cream and applied topically, BRL 39123 was as active as ACV against an HSV-1 infection of the dorsal skin in guinea pigs; it was also active topically (2% [wt/wt] in aqueous cream) in an HSV-2 genital infection in guinea pigs. Thus, it is clear that adequate concentrations of each compound were able to reach the epithelial cells in which the virus replicated. Larsson et al. (14) reported that 3HM-HBG (identical to BRL 39123) was active when applied topically at 5% (wt/wt) in aqueous cream to guinea pigs cutaneously infected with HSV-1 and that its activity equalled that of buciclovir. These researchers also reported that the compound was 140 times less active in guinea pig cells than in mouse cells, but despite this, it showed good topical activity in the guinea pig.

Before conducting experiments with BRL 39123 administered systemically to animals infected with HSV, we determined the concentrations of the compound in blood samples after its administration to mice. The concentrations of BRL 39123 and ACV in blood were comparable, i.e., 21 and 28 μg/ml, respectively, 15 min after a 50-mg/kg subcutaneous dose. After oral administration, however, concentrations of ACV in blood were up to 8-fold higher than those of BRL 39123. In mouse 3T3 cells, IC_{50}s for BRL 39123 and ACV against HSV-1(SC16) were 0.07 and 0.02 μg/ml, respectively; therefore, concentrations in blood were above the IC_{50}s for only about 3 h after oral or subcutaneous administration of either compound at 50 mg/kg.

An HSV-1 cutaneous infection in hairless mice was treated by systemic administration of the test compound. Subcutaneous treatment with either BRL 39123 or ACV resulted in activity superior to that after oral administration. Since the concentrations of BRL 39123 and ACV were much higher after subcutaneous doses than after oral doses, this finding suggests that high concentrations in blood are important for systemic treatment of HSV infections. BRL 39123 and ACV administered orally were equally active against this infection. Considering the relatively poor oral absorption of BRL 39123 and the lower susceptibility of HSV-1(SC16) to BRL 39123 in mouse cells, this finding is surprising but could be consistent with the accumulation of the phosphorylated form of BRL 39123 in infected cells.

In BALB/c mice with a cutaneous HSV-1 infection in the ear pinna, BRL 39123 and ACV administered subcutaneously reduced the lesion score in direct relation to the dose given, again pointing to the importance of concentrations in blood. Although treatment with BRL 39123 appeared to accelerate healing to a greater extent than ACV did, this difference was not statistically significant. We also showed that treatment with BRL 39123 commencing 5 h after infection was able to reduce the percentage of mice that developed latent infection from 90% in the untreated controls to a mean of 58% in the treated groups. However, in mice with a preexisting latent infection, administration of BRL 39123 in

TABLE 2. Effect of treatment with BRL 39123 or ACV on BALB/c mice infected intranasally with HSV

Virus strain	Dose schedule	Daily dose (mg/kg)	No. of survivors/group size			Mean survival time (days)[a]		
			BRL 39123	ACV	Vehicle	BRL 39123	ACV	Vehicle
HSV-1(SC16)	Single daily s.c.[b] dose, days 0 and 1	250	11/19	7/20	3/20	20**	10	8.5
	Single daily s.c. dose, days 0 to 6	100	10/10	7/10	3/10	∞***	33	11
	Drinking water, days 0 to 5	200	13/20	17/20	0/20	27***	88***	6.6
	Drinking water, days 1 to 5	200	11/20	9/20	0/20	18***	26***	6.6
HSV-2(10)	Single daily s.c. dose, days 0 to 6	100	14/20	8/20	5/20	31*	19	14
	Drinking water, days 0 to 7	200	0/20	6/20	0/20	10*	18***	8.7
	Drinking water, days 1 to 7	200	0/20	3/20	0/20	10*	15***	8.7

[a] Comparisons were made between treated and vehicle groups: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Comparisons between BRL 39123 and ACV were significant only in the groups infected with HSV-2 and treated with compounds in the drinking water ($P < 0.01$).

[b] s.c., Subcutaneous.

TABLE 3. Effect of subcutaneous administration of BRL 39123 and ACV on HSV-1(SC16) replication in DBA/2 mice after intraperitoneal infection with 5×10^5 PFU

Treatment	Times of administration (h after infection)	Mean infectivity titer of peritoneal wash (log_{10} PFU/0.1 ml) ± SD at the indicated time after infection (h)[a]					
		0.1	5	24	48	72	96
Vehicle	5, 24	4.35 ± 0.12	0.58 ± 0.34	3.07 ± 0.53	3.17 ± 0.14	1.02 ± 0.45	0.44 ± 0.28
BRL 39123 (50 mg/kg)	5	ND[b]	ND	0.30 ± 0[c]	0.60 ± 0.59[c]	0.30 ± 0	1.17 ± 0.76
	24	ND	ND	ND	0.30 ± 0	1.13 ± 0.54	0.91 ± 0.53
ACV (50 mg/kg)	5	ND	ND	2.81 ± 0.55	3.18 ± 0.48	1.44 ± 0.62	0.60 ± 0.59
	24	ND	ND	ND	1.61 ± 0.67	2.22 ± 0.51	0.69 ± 0.67

[a] Peritoneal washes were collected from five replicate animals and titrated individually.
[b] ND, Not done.
[c] $P < 0.01$ in a comparison between BRL 39123 and the corresponding ACV group.

drinking water for 14 days (100 mg/kg per day) failed to eliminate latent virus. Our results are similar to those of others using ACV (3, 12). These other researchers found that treatment initiated early after infection was able to prevent the development of latency but that treatment of an established latent infection was unable to eradicate the virus.

Two different systemic HSV infections in mice were treated with either BRL 39123 or ACV. With an intranasal infection, we showed that single daily subcutaneous dosing (100 mg/kg) for 7 days with BRL 39123 was more effective than similar dosing with ACV. Moreover, treatment for only 2 days with BRL 39123 at a higher dose (250 mg/kg) was also very effective. It has been shown (4) that the activity of BRL 39123 is more persistent than that of ACV in cell culture, and it was suggested that effective treatment with BRL 39123 might not be solely dependent on the maintenance of antiviral drug concentrations in the bloodstream. The data in the experiment described in Table 2 may be a consequence of this persistent activity, since after a single dose of BRL 39123 (100 mg/kg), concentrations in blood were higher than the IC_{50} for only about 3 h (Table 1). Thus, for the greater part of the 24 h after administration of BRL 39123, the concentrations in the bloodstream were below the IC_{50}s for HSV-1. Furthermore, results from an experiment in which mice were infected intraperitoneally with HSV-1 showed that a single dose of BRL 39123 given 5 h after infection was as effective as 3 equal doses of ACV given 1, 5, and 20 h after infection. In this mouse infection, BRL 39123 was also 10-fold more potent than ACV in preventing HSV replication. These results are unlikely to be due to differences in pharmacokinetics of the two compounds, since after a 50-mg/kg subcutaneous dose, the concentrations in blood were comparable (Table 1). It has been observed by others that ACV had to be administered every 6 h to achieve maximum efficacy in mice and that this requirement was related to the maintenance of adequate concentrations in blood (6). While we have shown in several animal models that initial concentrations of BRL 39123 in blood are important, the activity of the compound in the intraperitoneal model suggests that its activity is not dependent on the maintenance of high concentrations in blood and consequently can be administered less frequently.

In contrast to the results of Larsson et al. (14), who reported that 3HM-HBG (BRL 39123) supplied in drinking water was ineffective against mice infected intranasally with HSV-2, we have shown that BRL 39123 given in drinking water was active ($P < 0.05$) against HSV-2, although greater activity was noted against HSV-1. In this model, ACV was more active than BRL 39123 against HSV-2 when the compounds were administered in drinking water. We suggest that this result is related both to the greater potency of ACV against HSV-2 and to the maintenance of adequate levels in blood by this method of administration.

Other researchers (14, 15, 19) have reported that BRL 39123 was active in mice infected intraperitoneally with HSV, although mortality, rather than virus replication, was monitored. In addition, MacCoss et al. (15) showed that the activity was similar to that of ACV.

It has been proposed that the persistent antiviral activity of BRL 39123 and the related acyclic nucleosides buciclovir and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (4, 5, 14, 17, 18) may be due to the stability of the nucleoside triphosphate in infected cells. Studies on the biochemical mode of action of BRL 39123 have shown that the triphosphate of BRL 39123 within cells infected with HSV-1 has much greater stability than the triphosphate of ACV (M. R. Boyd, T. H. Bacon, C. Patience, D. Sutton, R. A. Vere Hodge, and M. Cole, Abstr. 7th Int. Cong. Virol. 1987, R32.6, p. 217). We suggest that the initial concentration of BRL 39123 in blood is important, since it determines the ultimate concentration of the antivirally active triphosphate within the infected cell. A high initial concentration in the blood is therefore vital. However, because of the relative stabilities of the triphosphates, we believe that the continuous maintenance of high concentrations in blood may be less important for BRL 39123 than for ACV. The results described in the present study support the view that BRL 39123 should have efficacy against herpesvirus infections in humans.

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