Pharmacokinetics and Antiviral Activity in Simian Varicella Virus-Infected Monkeys of (R,S)-9-[4-Hydroxy-2-(Hydroxymethyl) Butyl]Guanine, an Anti-Varicella-Zoster Virus Drug†

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The acyclic guanosine analog (R,S)-9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine, (±)2HM-HBG, is an effective inhibitor of herpes simplex virus and varicella-zoster virus infections *in vitro*. This report is concerned with the pharmacokinetic evaluation of the drug in rats and monkeys and its antiviral activity in African green monkeys infected with simian varicella virus (SVV), a virus closely related to varicella-zoster virus that is also susceptible to inhibition by (±)2HM-HBG. Elimination half-lives in plasma following intravenous administration to monkeys (100 µmol/kg of body weight) ranged from 1.8 to 2.2 h, and total body clearance was 9.0 ± 0.4 ml/min per kg (mean $\pm$ standard error). After oral administration, levels in plasma were low, with a maximum concentration of the drug of only 3.1 ± 0.8 µM, a time to reach maximum concentration of drug of 2.7 ± 0.4 h, and an oral bioavailability of $10.6 \pm 1.4\%$. Because of the low oral bioavailability, SVV-infected monkeys were treated intramuscularly with (±)2HM-HBG. (±)2HM-HBG at a dosage of 10 mg/kg of body weight per day allowed moderate viremia, whereas a dosage of 30 mg/kg of body weight per day strongly suppressed viremia with minimal numbers of virus plaques from blood specimens collected at days 3, 5, and 7 postinfection and complete clearance at day 9 postinfection. Titers of antibody to SVV were also low. Treatment three times daily was somewhat more efficacious than treatment twice daily. Thus, (±)2HM-HBG is an effective inhibitor of SVV replication *in vivo*, despite the fact that levels of (±)2HM-HBG in plasma were low at extended periods of time and below the concentration of drug giving 50% inhibition of plaque formation obtained *in vitro*.

The acyclic guanosine analog (R,S)-9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine, (±)2HM-HBG (Fig. 1), is an example of an anti-herpes simplex virus (HSV) drug activated by viral thymidine kinase and inhibiting viral DNA synthesis in infected cells (8). However, in contrast to its close analogs (Fig. 1), acyclovir, bucciclovir, 9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine, and ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine], (±)2HM-HBG is also a potent and selective inhibitor of varicella-zoster virus (VZV) replication (1). Thus, (±)2HM-HBG is an excellent and selective substrate for the VZV-induced thymidine kinase (1), and the triphosphate of the nucleoside is a potent and selective inhibitor of the viral polymerase (1).

When tested in HSV type 1 (HSV-1)- or HSV-2-infected mice, (±)2HM-HBG was less effective than bucciclovir in preventing death after the spread of HSV to the brain, despite the similar antiviral effects of bucciclovir and (±)2HM-HBG in cell culture (8). This limited efficacy of (±)2HM-HBG is due to the extremely short half-life and high clearance of the drug in plasma in mice (3, 8). This paper reports an investigation to determine the potential of (±)2HM-HBG in treatments of VZV-induced disease. We studied the pharmacokinetic profile of the drug in rats and monkeys, and on the basis of these findings, we investigated the efficacy of the drug in simian varicella virus (SVV)-infected African green monkeys. SVV is antigenically and

biochemically similar to human VZV (5, 10). Infections with SVV of the African green monkey have been useful in predicting the efficacy of drugs because the disease simulates VZV infection in immunocompromised hosts (10).

MATERIALS AND METHODS

Cell cultures and viruses. Rhesus monkey kidney (Ma 104) cells, African green monkey kidney (Vero and GMK) cells, and human embryonic lung (HL) fibroblast cells were grown in Dulbecco modified Eagle minimal essential medium with 10% heat-inactivated fetal calf serum. All cells were free of mycoplasma contamination.

An SVV strain isolated at the Delta Primate Center, Tulane University, Covington, La., was used to determine the amount of substance required for 50% plaque reduction. The SVV strain was cultured and passaged four times in tissue culture bottles (150 cm²; Costar, Cambridge, Mass.) prior to inoculation of monolayer cultures of cells in 24-well plates. No virus plaque purification was done for the SVV strain.

VZV strain Y was obtained from vesicles of a patient with clinical varicella infection and isolated at the National Bacteriological Laboratory, Stockholm, Sweden. The VZV strain was typed by using an enzyme-linked immunosorbent assay before each experiment (17) and was also subjected to virus plaque purification.

The effects of the drug on cell proliferation were studied as described previously (16), with the test compound being added to actively growing cells. The effects on cell numbers

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† Publication no. 98 from the Cooperative Antiviral Testing Group.

FIG. 1. Structural formulas of ($\pm$)2HM-HBG and some of its analogs mentioned in this report: buciclovir (BCV), 9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine (3HM-HBG), acyclovir (ACV), and ganciclovir (DHPG). The abbreviation 9G is used for the guanine base substituted in the 9-position with the indicated side chains.

were determined after 48 h with a cell counter (Analysinstrument AB, Stockholm, Sweden).

Plaque reduction assays. Confluent monolayers of Vero, GMK, Ma 104, and HL cells grown in 24-well plates (Costar) were infected with 230 to 9,000 PFU of SVV and 80 to 230 PFU of VZV. After an incubation period of 90 min at 37°C, the virus inoculum was removed and cells were washed with phosphate-buffered saline (PBS). A medium containing antiviral substances was then added, and the culture was incubated at 37°C in an atmosphere of 5% CO₂-95% air. Cultures were screened for plaques daily for 5 days with an Olympus microscope ($\times 80$ magnification). Plaques were quantified at day 5 when full plaque development occurred. After day 5, enlargement of plaques was noted, but the plaque number remained unchanged. No fixation or staining was needed for the quantification of plaques. Calculations of the inhibition of plaque numbers by 50% were done with reference to plaques formed in untreated SVV- and VZV-infected control cultures.

Viral sensitivity assay. Confluent HL cells in Costar 24-well plates were infected with 50 to 100 PFU of VZV strain 9/84 (National Bacteriological Laboratory), which was passaged more than 20 times in cell cultures and originally obtained from vesicles of a patient with clinical varicella. Following a 90-min period at 37°C in a humidified atmosphere of 5% CO₂-95% air, the inoculum was removed, cells were washed twice with PBS, and a medium containing an antiviral substance was added. The experiment was terminated at day 5 when a 50 to 70% cytopathic effect was noted in the virus-infected, untreated cells. Quantification of VZV antigens was done by an enzyme-linked immunosorbent assay (17, 18).

The sandwich assay for VZV antigens was carried out on microplates (Nunc, Roskilde, Denmark) coated with human zoster immunoglobulin (National Bacteriological Laboratory). The pooled, lysed infected-cell antigen from two to four wells was applied, followed by rabbit anti-VZV (20 μ g of immunoglobulin G per well) and alkaline phosphatase-labeled swine anti-rabbit immunoglobulin G (diluted 1:100 and applied at 100 μ l per well; Orion Diagnostica, Helsinki, Finland). The substrate was *p*-nitrophenyl phosphate (Sigma

Chemical Co.), and the reaction was read at 410 nm. To evaluate the inhibitory effect of ($\pm$)2HM-HBG, the optical densities of all samples were measured at 410 nm.

Monkeys. The African green monkeys used for infection studies were purchased as feral animals from a commercial source and were obtained principally from Somalia. All monkeys were quarantined for at least 90 days before use. They were wild-caught animals of mixed sex and age; their weights ranged from 2 to 6 kg. Efforts were made to select monkeys for treatment groups so that sex ratios and mean weights were similar for all groups. During early quarantine and immediately before release from quarantine, sera were collected and determined to be free of antibody to SVV by a plaque reduction assay (13). Male cynomolgus monkeys weighing around 3 kg were used for the pharmacokinetic studies.

Drug. ($\pm$)2HM-HBG was synthesized by the reaction of 2-amino-6-chloropurine and dimethyl itaconate in dimethylformamide with a catalytical amount of sodium hydride, followed by reduction with lithium borohydride in *tert*-butyl alcohol and hydrolysis by 70% aqueous formic acid. The drug was dissolved in PBS for both intravenous and oral studies.

Plasma pharmacokinetics. The rats (male; Sprague-Dawley; 250 g) and monkeys were fasted overnight but were allowed water before each study. The drug was administered at a dose of 100 μ mol/kg of body weight. Blood samples were taken, and plasma was obtained by centrifugation, deproteinized, and stored at -20°C until analysis. ($\pm$)2HM-HBG in plasma was determined by reversed-phase high-pressure liquid chromatography that incorporated a coupled-column technique (Lindkvist, Edlund, and Nordgren, Astra Alab AB, unpublished data). Deproteinized plasma samples were injected into the first column (C₁₈ microsphere; pore size, 3 μ m; dimensions, 100 by 4.6 mm) and eluted with phosphate buffer (μ = 0.1; pH = 2.0) at a flow rate of 1.0 ml/min. The fraction containing ($\pm$)2HM-HBG was trapped in a loop for subsequent injection and separation from endogenous material on a second C₁₈ column. The eluant was similar to that used in the first column except that it contained 25% methanol with 2 mM decylsulfate. The flow rate was also 1.0 ml/min. ($\pm$)2HM-HBG was detected by fluorescence at 370 nm after excitation at 280 nm.

Data for concentration in plasma versus time for ($\pm$)2HM-HBG were subjected to model-independent and model-dependent analyses. The areas under the curves of concentration in plasma versus time (AUC) were calculated by the trapezoidal rule and extrapolated to infinity. The terminal-phase half-life ($t_{1/2\beta}$) was calculated by linear regression with unweighted data. Total body clearance, mean residence time, volume of distribution, and bioavailability were calculated from appropriate equations.

Efficacy studies. Monkeys that were earlier determined to be free of antibody to SVV were moved from the quarantine holding area to an infectious-disease building and allowed to acclimate to their new surroundings for 1 week before the experiment was started. Base-line measurement of hematological and chemical parameters was performed 5 days before virus inoculation and again on the day of virus inoculation. Chemical parameters included whole-blood urea nitrogen and levels of creatinine, albumin, globulin, total protein, total bilirubin, aspartate aminotransferase, and alanine aminotransferase in serum.

Virus inoculation was performed as previously described (12). To produce consistent clinical infection with development of disseminated rash and measurable viremia, a dilu-

tion of stock SVV containing approximately 10^5 PFU was administered in a 3-ml volume of Eagle minimal essential medium with 10% fetal bovine serum, 1.5 ml by intratracheal catheter and 1.5 ml by subcutaneous inoculation into the abdominal area. The virus inoculum was titrated at the time of inoculation. The treatment with (±)2HM-HBG was begun after virus inoculation at the times described for each experiment. Treatment was given twice daily at 8.00 a.m. and 2.30 p.m. or three times daily at 8.00 a.m., 2.30 p.m., and 7.30 p.m. throughout the treatment period.

For the experiments reported in this paper, treatment was by either the intravenous or intramuscular route. In each experiment, blood was drawn for measurements of hematological and clinical chemical parameters and virus titer on days 3, 5, 7, 9, and, occasionally, 11 postinfection. The level of virus circulating in the blood and characteristically associated with lymphocytes was determined by layering 3 ml of heparinized blood over a Ficoll-Hypaque gradient. After centrifugation, the lymphocytes were collected, washed twice on RPMI 1640 medium containing 20% fetal bovine serum, and suspended in 10 ml of medium that was divided between two 25-cm² culture flasks seeded with Vero cells. Daily microscopic examination was performed on the inoculated cultures, which were fixed in methanol and stained with methylene blue-basic fuchsin when plaques were visible and of sufficient size for counting. The number of plaques in each flask was counted, and the mean number for the two cultures from each specimen was recorded.

Monkeys were examined daily for evidence of clinical disease and specifically for the appearance of a rash. A rash was scored on a scale of ± to 4+, with 4+ indicating numerous vesicles disseminated over the entire body and ± indicating minimal and very localized vesicle distribution. Monkeys that died as a consequence of severe disseminated simian varicella infection were given a complete necropsy, including histologic examination of involved tissues.

At 14 and 21 days after virus inoculation, blood was drawn for the determination of the titer of antibody to SVV (14). Twofold dilutions of heat-inactivated sera were mixed with an equal volume of medium containing SVV at a concentration of 100 to 200 PFU in the final dilutions.

After 1 h of incubation at room temperature, the serum-virus mixtures were plated in duplicate in petri dishes containing Vero cells. After incubation at 37°C in a CO₂ incubator until the development of virus plaques, the cultures were fixed and stained, and the number of plaques was counted. The titer of antibody was the maximum dilution of serum that inhibited 80% or more of the plaques present in control cultures without serum.

RESULTS

Pharmacokinetic studies. Previous studies of mice had indicated the poor systemic availability of (±)2HM-HBG (8). The experiments with rats confirm the findings of poor absorption in mice. The use of sodium and hydrochloride salts was not enough to improve oral bioavailability (Table 1). (±)2HM-HBG was administered to four cynomolgus monkeys intravenously as a single bolus and orally by a stomach tube in order to determine its pharmacokinetic profile. Figure 2 depicts the profiles of concentration in plasma versus time. The curve obtained after intravenous administration is best described as biexponential with an initial rapid decline followed by a slower terminal phase. After oral dosing of the animals, peak levels of the compound in plasma were obtained on average after about 3 h,

TABLE 1. Oral bioavailability of (±)2HM-HBG and its sodium and hydrochloride salts in rats^a

Compound	$C_{\max}$ (μM)	$T_{\max}$ (h)	Oral bioavailability (%) ^b
(±)2HM-HBG	0.7 ± 0.1	0.8 ± 0.1	11.5 ± 2.1
(±)2HM-HBG · Na	0.6 ± 0.1	2.3 ± 1.1	22.8 ± 8.3
(±)2HM-HBG · HCl	1.8 ± 1.3	0.6 ± 0.1	8.2 ± 0.7

^a Values are means ± standard errors for five animals. $C_{\max}$, Maximum concentration in plasma; $T_{\max}$, time to reach maximum concentration in plasma.

^b Calculated as the ratio of the AUC after oral dosing to that after an intravenous bolus.

whereupon the rate of elimination paralleled that obtained after intravenous dosing. Relevant parameters are given in Table 2. These results confirm the low degree of absorption of the compound and preclude the study of its efficacy by using oral administration.

Antiviral effects in monkey kidney cell lines. Replication of SVV and VZV was inhibited by (±)2HM-HBG. The concentrations of (±)2HM-HBG required for 50% inhibition of plaque formation (IC_{50}) of SVV ranged from 35 to 91 μM (Table 3). The plaque formation of VZV in Vero and Ma 104 cells was inhibited to the same extent at 2 to 4 μM (Table 3). Whereas the treatment of VZV-infected HL cells with 10 μM (±)2HM-HBG for 5 days resulted in 82% inhibition of virus replication, as determined by the viral sensitivity assay, a similar extent of inhibition (73%) was obtained when the cultures were treated with 10 μM of (±)2HM-HBG for 5 days, three times daily. Each treatment lasted for only 3 h. This result was also observed at drug concentrations of 2 and 5 μM (results not shown) and indicates that (±)2HM-HBG can be given intermittently to obtain antiviral effects (see below). The selectivity index (the ratio of drug concentration giving 50% inhibition of cell growth to IC_{50}) of (±)2HM-HBG in Vero cells is much lower for SVV than for VZV (27 and 465, respectively). However, as shown earlier for the anti-HSV effects of bucciclovir in guinea pigs, a limited effect in cell culture does not exclude potent antiviral effects in vivo (8).

Effects in SVV-infected monkeys. Because of the low oral bioavailability of (±)2HM-HBG, nine African green monkeys were selected for an experiment to evaluate the antiviral activity of (±)2HM-HBG administered by intramuscular or intravenous injection. Each of the nine monkeys was infected by intratracheal and subcutaneous inoculations of SVV administered as a total dose of 1.0×10^6 PFU per monkey. Treatment with (±)2HM-HBG was begun 48 h after virus inoculation, and the drug was administered at 25 mg/kg of body weight per day in divided doses twice daily. A control group received injections of PBS on a similar schedule.

All monkeys were observed daily for evidence of clinical disease. Each of the three control monkeys developed infection with viremia and a rash (Table 4). One control monkey died with severe simian varicella. The remaining two control monkeys exhibited minimal viremia with moderate to severe rash. The rash persisted for 5 to 7 days. Antibody to SVV was present in high titer at 14 days postinfection.

Monkeys treated with (±)2HM-HBG by either intramuscular or intravenous injection exhibited minimal viremia on each of the sampling days. Viremia, while present, was less than that seen in the untreated control monkeys. Treatment prevented the development of a rash. Only one monkey

FIG. 2. Curves of concentration in plasma versus time for oral (○) and intravenous (□) administration of (±)2HM-HBG in male cynomolgus monkeys. The animals were each given a single dose of 100 μmol/kg of body weight intravenously and orally on separate occasions. Each point of data is the mean value for four animals. Error bars represent a 90% confidence interval.

(G667) in the group receiving drug by intravenous injection developed a very minimal rash that persisted for 2 days. Titers of antibody to the virus were lower for the drug-treated monkeys than for the control monkeys.

We then evaluated dosages of 30 and 10 mg/kg of body weight per day given by intramuscular injection three times daily, starting at 48 h postinfection and continuing for 10 days. The infection dose was 2.1×10^5 PFU of virus per monkey. Each of the three control monkeys developed a rash, and two of three control monkeys died with disseminated simian varicella infection (Table 5). Of the three monkeys treated with 10 mg/kg of body weight per day, one monkey developed a moderately severe rash, whereas the remaining two monkeys each developed an extremely minimal rash with only one or two vesicles observed that promptly cleared after 1 day. The monkeys receiving 30 mg/kg of body weight per day did not develop any rash throughout the 14-day observation period. Viremia was detected in each of the three control monkeys, with two monkeys showing very high concentrations of virus in the circulating lymphocytes. These two monkeys subsequently died. The third control monkey also developed a marked viremia on day 7 postinfection. The dosage of 10 mg/kg of body weight per day allowed moderate viremia on day 7 postinfection in

each of three monkeys, with minimal viremia on the other days of sampling. (±)2HM-HBG at 30 mg/kg of body weight per day strongly suppressed viremia, with minimal numbers of virus plaques from blood specimens collected at 3, 5, and 7 days postinfection, which completely cleared by day 9. Titers of antibody to SVV were low in the monkeys receiving 30 mg of (±)2HM-HBG per kg of body weight per day and were moderate to high in the monkeys receiving 10 mg/kg of body weight per day. The low titers presumably reflect a lowered antigenic stimulus dose to virus inhibition by the drug.

Thus, (±)2HM-HBG appears to be an effective inhibitor of SVV replication in virus-inoculated monkeys. The data suggest that treatment given by intramuscular injection three times daily is somewhat more efficacious than treatment twice daily.

Toxicity in vivo. Hematologic and clinical chemical tests were done twice prior to virus infection and at 3, 7, 9, and 11 days after virus inoculation. Clinical chemical tests included the determinations of aminotransferases, blood urea nitrogen, creatinine, albumin, globulin, A/G ratios, and total protein. No obvious toxicity was indicated by the hematologic and clinical chemical tests (data not shown).

TABLE 2. Pharmacokinetic parameters for (±)2HM-HBG in male cynomolgus monkeys^a

Monkey	Result after oral administration				Result after intravenous administration				Oral bioavailability (%) ^b
	C_{max} (μM)	T_{max} (h)	$AUC_{0-\infty}$ (μM · h)	$t_{1/2\beta}$ (h)	MRT (h)	CL (ml/min per kg)	V (liter/kg)	$AUC_{0-\infty}$ (μM · h)	
A	1.5	4.0	15.0	1.8	1.3	8.0	1.28	208.8	7.0
B	5.6	2.0	18.2	2.2	1.5	9.8	1.85	170.1	10.5
C	2.5	2.0	19.6	1.6	1.3	9.4	1.35	177.1	11.0
D	3.0	3.0	26.0	1.8	1.4	8.9	1.42	186.4	13.8
Mean ± SE	3.1 ± 0.8	2.7 ± 0.4	19.7 ± 2.3	1.8 ± 0.1	1.4 ± 0.05	9.0 ± 0.4	1.47 ± 0.25	185.6 ± 8.4	10.6 ± 1.4

^a C_{max} , Maximum concentration in plasma; T_{max} , time to reach maximum concentration in plasma; $AUC_{0-\infty}$, AUC from time 0 to infinity; $t_{1/2\beta}$, terminal-phase half-life; MRT, mean residence time; CL, total body clearance; V, apparent volume of distribution.

^b Calculated as the ratio of the AUC after oral dosing to that after an intravenous bolus.

TABLE 3. Effects of (±)2HM-HBG in cell cultures^a

Cell type	IC ₅₀ (μM)		TC ₅₀ (μM) ^b
	SVV	VZV	
Vero	35 ± 22	2.0 ± 1.0	930 ± 250
GMK	91 ± 8	— ^c	
Ma 104	83 ± 11	3.7 ± 0.5	870 ± 35
HL	—	4.2 ± 0.6	

^a Values are means ± standard deviations that were determined from at least three dose-response curves with eight different concentrations of the drug.

^b TC₅₀, Concentration of drug giving 50% inhibition of cell growth.

^c —, Cells could not be productively infected with the indicated virus.

DISCUSSION

The oral bioavailability of (±)2HM-HBG in monkeys and rats (Tables 1 and 2) and in mice (3, 8) is low. We observed similar low oral bioavailabilities (≤20%) for bucciclovir in mice (8) and in humans (Astra Alab AB, unpublished data) and for 9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine and 9-(4-hydroxybutyl)guanine in mice (4, 8). To determine the potential of (±)2HM-HBG in SVV-infected monkeys, we, therefore, chose to administer the drug intravenously or intramuscularly.

A beneficial effect of intramuscular or intravenous administration of (±)2HM-HBG on disease development and viremia in SVV-infected monkeys was observed. The dosage for therapeutic antiviral activity of (±)2HM-HBG administered intramuscularly was between 10 and 30 mg/kg of body weight per day. The effective dosage of (±)2HM-HBG must be, therefore, lower than those of ganciclovir and acyclovir (12, 13).

The antiviral activity of (±)2HM-HBG in vivo is surprising, because levels of (±)2HM-HBG in plasma exceeded the IC₅₀s obtained for (±)2HM-HBG in cultures of SVV-infected monkey kidney cells for a period not exceeding 0.5 (twice a day) or 0.8 (three times a day) h after injection. Two explanations, which are not mutually exclusive, are possible. First, in vivo, the guanosine analog is accumulated

intracellularly in infected cells in a phosphorylated form, which is the actual inhibitor of viral DNA synthesis (1). This case has been observed earlier with bucciclovir in HSV-1- or HSV-2-infected mice (3, 4, 8). These results showed that the antiviral effect persisted even with drug levels in plasma below the IC₅₀s obtained for HSV-infected cell cultures because of the low metabolic turnover of the triphosphate of the guanosine analog, which is the antivirally active agent (3, 11, 15). In cell cultures also, treatment with bucciclovir for a short period is sufficient to obtain an antiviral effect (15), and this condition also applies to (±)2HM-HBG, as shown by the present study. A critical determinant of this so-called trapping phenomenon is likely the initial rate of phosphorylation of the nucleoside analog (9, 11). Indeed, (±)2HM-HBG is an excellent substrate for the VZV-coded thymidine kinase (2) and possibly also for the SVV-coded thymidine kinase.

A second explanation for the in vivo activity of (±)2HM-HBG is that the IC₅₀s obtained for monkey kidney cells in vitro underestimate the antiviral effect in monkey lymphocytes in vivo. Monkey kidney cells can maintain a high endogenous pool of deoxythymidine, which interferes with the phosphorylation of acyclic guanosine analogs (2, 6, 7). Furthermore, exogenously added deoxythymidine can antagonize the antiviral effect of (±)2HM-HBG (2). Our attempts to productively infect monkey lymphocytes with SVV in vitro were, however, unsuccessful. Therefore, we could not determine the IC₅₀s for (±)2HM-HBG in these cells.

The large apparent volumes of distribution (>1 liter/kg) observed for both rats and monkeys indicate that the compound was distributed to tissues in these animals. Moreover, values for this parameter were fivefold greater in rats than in monkeys. In addition, total clearance values were also about fivefold greater in rats. These results indicate that the rapid removal of the drug from circulation (clearance) in rats reflects extensive metabolic conversion, which results in increased affinity for tissues. This affinity for tissues, in turn, is reflected in the large volume of distribution observed.

Total clearance in mice is of the same order of magnitude as that in rats, although the volume of distribution in mice is only 1/10 of that in rats (8). Presumably, clearance in mice is

TABLE 4. Effect of mode of administration of (±) 2HM-HBG on SVV infection in African green monkeys

Treatment ^a and monkey no.	Viremia (mean PFU) ^b on indicated day postinfection				Severity ^c of rash on indicated day postinfection								Antibody titer ^d	
	3	7	9	11	6	7	8	9	10	11	12	14	14 days	21 days
Intramuscular														
G272	6.5	2	4	2									1:320	1:320
F751	4	4.5	1.5	4									1:20	1:40
G666	12	4	2.5	4									1:40	1:80
Intravenous														
G667	6	2.5	5.5	1.5		±	±						1:160	1:160
F725	8	3.5	2.5	7									1:40	1:80
G673	2.5	3.5	6.5	2.5									1:80	1:80
Control														
F644	38.5	>400	>400	— ^e	+	3+	4+	4+						
G668	8	32	29	25.5			±	2+	+	±	±		1:320	1:640
G664	24.5	16.5	39	34.5		+	3+	4+	4+	2+	+	+	>1:320	≥1:640

^a Treatment with (±)2HM-HBG at 25 mg/kg of body weight per day was started 48 h after virus inoculation and continued for 10 days. Monkeys in the control group were given PBS.

^b Viremia was expressed as mean number of viral plaques present in two Vero cell cultures inoculated with lymphocytes separated from a 3-ml blood sample on the indicated days postinfection.

^c A rash was scored daily on a scale of ± to 4+.

^d Titers of antibody were expressed as the dilutions of serum causing an 80% or greater reduction of PFUs in control cultures without added serum.

^e —, The animal died on day 10 postinfection.

TABLE 5. Effect of ($\pm$)2HM-HBG dosage on SVV infection in African green monkeys

Dosage (mg/kg of body wt. per day) ^a and monkey no.	Viremia (PFU) ^b on indicated day postinfection					Severity of rash ^b on indicated day postinfection								Antibody titer ^b	
	3	5	7	9	11	7	8	9	10	11	12	13	14	14 days	21 days
30															
H118	1	1	9	0	0									1:10	1:20
H117	1	3	7	0	0									<1:10	1:20
H116	1	3	8	0	0									1:10	1:20
10															
H114	1	3	46	10	0			1+	3+	3+	2+	1+	±	1:160	1:320
H113	0	6	45	1	0				±					1:20	1:80
H132	1	1	20	0	0				±					1:40	1:640
0															
H130	1	18	130	31	0			1+	2+	3+	3+	3+	2+	1:320	>1:640
H129	2	82	>1,000	>1,000	— ^c	±	1+	2+	2+	—					
H128	2	50	>1,000	>1,000	—		1+	2+	—						

^a The drug was administered by intramuscular injection beginning at 48 h after virus inoculation, and the treatment was continued for 10 days. Monkeys in the control group (0 mg/kg of body weight per day) were given PBS.

^b See Table 4, footnotes *b* to *d*, for explanations.

^c —, The monkeys died on day 11 (H129) and day 10 (H128).

mainly due to nonmetabolic processes. This type of clearance may explain the ineffectiveness of the compound as an antiviral agent in mice (3, 8). The comparatively lower clearance in monkeys and a volume of distribution greater than 1 liter/kg may contribute to the favorable antiviral effect of ($\pm$)2HM-HBG in monkeys. Provided that these pharmacokinetic parameters are similar for both monkeys and humans, ($\pm$)2HM-HBG may be an effective antiviral drug against generalized VZV infection in humans.

ACKNOWLEDGMENTS

We gratefully acknowledge the expert help of A. Persson and S. O. Lindkvist with the high-pressure liquid chromatographic analyses and of M. Kropp with the preparation of the manuscript.

A portion of these studies was supported by Public Health Service grant NO-AI-62521 from the National Institutes of Allergy and Infectious Diseases Antiviral Substances Program.

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