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Pharmacokinetics of Michellamine B, a Naphthylisoquinoline Alkaloid with In Vitro Activity against Human Immunodeficiency Virus Types 1 and 2, in the Mouse and Dog

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Michellamine B (MB) is a naturally occurring naphthylisoquinoline alkaloid of novel chemical structure with activity against human immunodeficiency virus (HIV) types 1 and 2 in vitro. In conjunction with its preclinical evaluation, the plasma pharmacokinetics of MB was characterized in mice and dogs treated by intravenous infusions of 1- and 15-min durations, respectively. At doses ranging from 1 to 9 mg/kg of body weight, the drug exhibited apparent first-order kinetics in both species, affording triexponential plasma concentration-time profiles. Treatment with doses of 5 to 9 mg/kg provided peak plasma levels within the range that completely inhibits the cytopathic effects of HIV upon cultured human lymphoblastoid cells (50 to 100 μg/ml) without evidence of toxicity. MB had a biological half-life of 2.8 ± 0.8 h in mice, with a mean residence time of 2.1 ± 0.3 h, and a total plasma clearance of 2.4 ± 0.5 ml/min/kg (mean ± standard deviation; $n = 3$); however, the terminal-phase contribution to the area under the plasma profile from time zero to infinity was 44.6% ± 12.9%. In contrast, the terminal phase was the primary determinant of drug disposition in dogs, accounting for 74.1% ± 2.8% ($n = 3$) of the area under the curve. Furthermore, the systemic duration of MB was significantly longer in the dogs than in mice, as indicated by mean values of the apparent half-life (11.6 ± 1.2 h), mean residence time (12.3 ± 1.8 h), and clearance (0.50 ± 0.08 ml/min/kg). However, there was no statistical difference between its apparent volume of distribution in the mice (0.60 ± 0.08 liters/kg) and dogs (0.50 ± 0.07 liters/kg). A single dog was also treated with a total dose of 97 mg/kg given as a 72-h constant-rate intravenous infusion, since prolonged systemic exposure to potentially therapeutic drug concentrations will very likely be required for clinical anti-HIV effects. Within 4 h after starting the infusion, the plasma MB concentration exceeded 18 μg/ml, its reported 50% effective concentration against HIV in vitro, and subsequently increased to 41 μg/ml at the end of the infusion. There were no clinical or pathological indications of toxicity. Whereas the total plasma clearance (0.48 ml/min/kg) was within the range observed for dogs treated by 15-min infusion, extension of the postinfusion sampling period from 24 h to 4 days facilitated better definition of the terminal exponential phase, yielding a value of 25.6 h for the biological half-life of MB. The amount of drug excreted by dogs unchanged in the urine ranged from 3.7 to 11.1% of the administered dose. Thus, the major pathways by which the drug is eliminated from the body remain to be identified. On the basis of these findings, continued development of MB as a novel lead compound for the treatment of HIV infection is warranted.

The activity of michellamine B (MB) against human immunodeficiency virus (HIV) was discovered in the in vitro screening program established by the National Cancer Institute to identify new chemotherapeutic agents that may prove effective for the treatment of AIDS (2, 11). MB is a naturally occurring compound that represents a previously unknown dimeric variant of the naphthylisoquinoline alkaloids produced by the Ancistrocladaceae family of tropical plants. Its chemical structure is shown in Fig. 1 (3). While the mechanism underlying the antiretroviral activity of MB has not been reported, it inhibits the replication of both HIV type 1 (HIV-1) and HIV-2, as well as their associated cytopathic effects upon cultured human T-lymphoblastoid cells (lines CEM-SS and MT-2), with a 50% effective concentration near 18 μg/ml (11). Complete protection from viral cytopathicity is afforded at concentrations three- to fourfold lower than the 50% inhibitory concentration for drug-induced cytotoxicity (180 μg/ml), representing a moderate therapeutic index. The demonstration of similar in vitro potencies against HIV-1 and HIV-2 holds promise that MB will not prove susceptible to the therapeutic deficiencies characteristic of the nonnucleoside reverse transcriptase inhibitors evaluated to date (9). Although inactive against HIV-2, these agents are generally quite potent against HIV-1 in vitro, with impressive therapeutic indices. Unfortunately, they have been found to be clinically ineffective against HIV-1 because of rapid mutation of the molecular target, rendering the virus resistant not only to the particular drug to which it was originally exposed but also to structurally distinct compounds that function in the same manner.

A preliminary pharmacokinetic evaluation of MB in mice demonstrated that a dose of 19 mg/kg of body weight administered subcutaneously provides peak concentrations in plasma approaching 10 μg/ml at 1 h postinjection (14). With an apparent mean residence time (MRT) of 33.7 h, the concentration of drug in plasma decreases by only a factor of 5 from the peak value during 24 h. While there were no perceptible indications of toxicity, the volume of solution required to deliver the predicted dose to maintain optimal therapeutic concentra-

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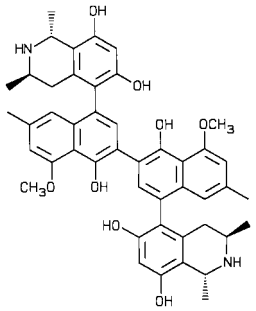

FIG. 1. Chemical structure of MB.

tions in plasma, as indicated by the in vitro anti-HIV studies, would be impractical for the subcutaneous route of administration. Consistent with the physicochemical properties of MB (11, 14), which probably exists in a charged state throughout the gastrointestinal tract, the oral bioavailability in mice is essentially negligible (14). Consequently, the intravenous (i.v.) route presents the only obvious alternative for the administration of MB. The development of a parenteral formulation should not prove problematic, as the drug appears to be adequately soluble and chemically stable under compatible conditions (14). The present study was therefore undertaken with the objective of characterizing the disposition of MB in mice and dogs.

MATERIALS AND METHODS

Drug formulation. The diacetate salt of michellamine B (NSC 649324) was provided by the Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. The drug was formulated for injection by dissolving it in a vehicle composed of propylene glycol-normal saline for injection, USP (1:9, vol/vol) adjusted to a pH of ~3 with phosphoric acid. Solutions were prepared with drug concentrations of 0.5 to 5 mg/ml for the mouse studies and 1 to 10 mg/ml for the dog studies. They were stored at −20°C until used. Solution densities were estimated by weighing three 10-μl aliquots, measured with a Hamilton (Reno, Nev.) syringe, on a C-31 microbalance (Cahn Instruments, Cerritos, Calif.). The concentration of MB in each dosing solution was ascertained by high-performance liquid chromatography (HPLC) as described below.

Dosing and sample collection. Male Harlan CD2F$_1$ mice, weighing 23.5 ± 1.8 g (mean ± standard deviation [SD]; n = 144), that had not been subjected to fasting were treated with 0.98, 4.74, and 9.49 mg of MB per kg of body weight by 60-s tail vein injection with a 26-gauge, 3/8-inch (ca. 9.5-mm) Precision Glide Needle (Becton Dickinson, Rutherford, N.J.) without anesthesia. The animals were given free access to food and water. At 16 time intervals, ranging from 3 min to 8 h postinjection, three mice were anesthetized with methoxyfluorane and bled by retro-orbital puncture with heparinized Pasteur pipettes. Each blood specimen was centrifuged (12,000 × g, 5 min, 25°C) immediately upon collection. Plasma was separated from the pelleted blood cells, flash frozen, and stored at −20°C until it was assayed.

Female beagle dogs (Hazelton Research Products, Kalamazoo, Mich.) were given 0.88, 4.95, 8.63, and 24.0 mg of MB per kg of body weight by 15-min constant-rate i.v. infusion at the Frederick Research Center/Southern Research Institute (Frederick, Md.). A single animal was treated at each dose level. The animals were fully acclimated and given a complete physical examination prior to approval for use by an attending veterinarian. They were subjected to fasting from the evening before to 8 h after receiving the infusion. During the study, the median age of the dogs was 13 months (range, 12 to 14 months) and their mean weight was 9.25 kg (range, 9.1 to 9.4 kg). The dogs were given 0.5 mg of atropine (Fort Dodge Laboratories, Fort Dodge, Iowa) by intramuscular injection to control salivation before light sedation with i.v. thiamylal sodium (Bio-Ceutic, St. Joseph, Mo.) 1 to 2 h prior to the administration of MB. A percutaneous 17-gauge IV Intrafusor with an 18-gauge 11.4-cm catheter (Sorenson Research, Salt Lake City, Utah) was placed into a saphenous vein for introduction of the dosing solution. A 17-gauge CVP Intrafusor with an 18-gauge 53.3-cm catheter (Sorenson Research) was inserted percutaneously into the other saphenous vein for blood sampling. Sterile 0.9% sodium chloride (Abbott Laboratories, Chicago, Ill.), used throughout as a catheter flushing solution, was delivered at ~10 ml/h with a Cormed ML6-8 infusion pump (Dakmed, Buffalo, N.Y.) to maintain patency of the venous catheters. After insertion of a Buster 8F urinary catheter (2 by 500 mm; A. J. Buck & Sons, Owings Mills, Md.), the dog was placed in a sling (Alice King Chatham Medical Arts, Los Angeles, Calif.) and permitted to recover from the anesthetic.

An aliquot of blood (10 ml) was collected to afford pretreatment plasma and the urinary bladder was flushed with saline before the contents were drained via the catheter. Subsequently, the dosing solution was delivered from a weighed 65-ml IPR-86 infusion bag (Cardio Medical Products, Rockaway, N.J.) into the venous catheter at a constant rate for 15 min with a Cormed ML6-8 infusion pump. By way of a three-way stopcock, normal saline was then introduced into the infusion line at an identical flow rate to pass the remaining solution into the vein. The time at which the dose actually entered the vein was established from previous determinations of the flow rate of the pump and volume within the infusion line and catheter. The infusion bag was reweighed to determine the amount of solution delivered.

Pharmacokinetic blood specimens (1.5 ml) were drawn into heparin-treated syringes at 22 time intervals during the infusion and subsequent 8 h. The urinary bladder was drained every 2 h, or more frequently as required, and was flushed with saline (2 to 3 ml) until colorless. After the 8-h specimens were collected, the catheters were removed and the animal was housed in a standard stainless steel cage with a wire mesh floor and a urine collection tray (Allentown Caging Equipment, Allentown, N.J.). Pooled urine was collected at 12 and 24 h; another blood sample was obtained at 24 h postinfusion. Blood samples were promptly centrifuged (1,800 × g, 10 min, 25°C) to provide plasma, which was handled as described above. The volume and pH of the urine and the combined volume of the recovered bladder flushes were measured. Plasma and urine specimens were kept frozen at −20°C until assayed. Except in the 0.88-mg/kg-dose study, blood samples were acquired for clinical pathology evaluations several days prior to dosing and periodically for 2 weeks posttreatment. The tests performed included a standard hematology profile with differentials, reticulocyte count, platelet count, and prothrombin time. Liver function tests included determinations of glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, alkaline phosphatase, total protein, and total bilirubin in serum. Other serum measurements included lactic dehydrogenase, creatine phosphokinase, glucose, urea nitrogen, creatinine, sodium, potassium, and chloride (AniLytics, Gaithersburg, Md.; Maryland Medical Laboratory, Baltimore, Md.).

A single female beagle dog was also treated with a total dose of 97.0 mg/kg given as a 72.0-h continuous i.v. infusion at the Southern Research Institute (Birmingham, Ala.). The average weight of the dog during the infusion period was 7.33 ± 0.25 kg (mean ± SD; n = 3). The dosing solution (10.61 mg/ml) was delivered at a constant rate of 0.127 ± 0.006 ml/kg/h, with a battery-operated Walkmed 410 ambulatory infusion pump (Medfusion, Duluth, Ga.), into a percutaneous Intrafusor catheter fully advanced through the jugular vein. The drug reservoir bag and pump batteries were replaced every 24 h during the infusion period. Blood samples for plasma drug level monitoring were drawn through a heparin lock attached to a CVP Intrafusor catheter advanced through the femoral vein into the superior vena cava. Specimens (1 ml) were acquired at 26 time intervals during treatment, beginning 3 min after the infusion was started, and at 24 points from 3 min to 96 h postinfusion. Urine was collected by drainage of a bladder catheter as required and pooled at 12-h intervals during the infusion and the following 4 days. In addition, blood samples for clinical pathology tests were drawn before treatment and periodically for 3 weeks thereafter.

Drug level monitoring. The concentration of MB in plasma and urine specimens was determined by an HPLC assay (14). Briefly, the samples (50 μl) were prepared for analysis by initial treatment with dilute trichloroacetic acid (5 μl, 0.5 M) and mixing on a vortex stirrer for 15 s. A solution of the internal standard in acetonitrile (200 μl of α-naphthoflavone at a concentration of 0.75 μg/ml) was added. The resulting mixture was vortexed for 30 s and centrifuged (12,000 × g, 5°C, 10 min). The supernatant (175 μl) was separated from the pellet and diluted with 250 μl of the aqueous component of the mobile phase. A 250-μl aliquot of this solution was loaded onto a 3.9-mm by 15-cm column of 4 μm Nova-Pak Phenyl (Waters Associates, Milford, Mass.). Chromatography was performed at ambient temperature, using an isocratic mobile phase composed of 10 mM octyl sodium sulfate and 15 μM tetrabutylammonium hydrogen sulfate in acetonitrile–0.05 M ammonium formate buffer, pH 4.0 (46:54, vol/vol), at a flow rate of 0.6 ml/min. Elution of the components of interest was monitored by fluorescence emission at 393 nm, with 237 nm as the excitation wavelength.

Quantitation was performed by similarly assaying a series of eight plasma or urine standards with known concentrations of the analyte (0.01 to 1.0 μg/ml) and a drug-free sample. Standard curves were constructed by plotting the chromatographic peak area of MB relative to the internal standard against MB concentration. Linear least-squares regression, with a weighting factor of $1/y_{obs}$, without inclusion of the origin was performed to determine the slope, y intercept, and correlation coefficient of the best-fit line. MB concentrations in unknown samples were calculated by using the results of the regression analysis. Specimens with concentrations exceeding the upper range of the standard curve were reassayed upon appropriate dilution with drug-free plasma or urine. All samples were initially assayed in duplicate, and if the replicate determinations deviated from the average by more than 10%, additional analyses were performed. During a period of 11 months, the lowest concentration of MB included in the plasma standard curves, 0.010 μg/ml, was quantified with a coefficient of variation of less than 10% (14).

Pharmacokinetic data analysis. Actual doses were calculated from the body weight of the animals on the treatment day, the volume of dosing solution delivered, and the assayed concentration of MB in each preparation. The beginning and ending times of the drug input and sample collection intervals were monitored with a digital timer and recorded to the nearest second. Time points were calculated as the difference between the midpoint of the blood collection interval and the starting time of dose administration. For the studies of disposition in mice, the geometric mean plasma MB concentration was calculated from the observed concentrations for three animals at each time point.

Plasma concentration-time profiles were pharmacokinetically analyzed by noncompartmental methods (7). Conventions recommended by Rowland and Tucker for symbols of pharmacokinetic terms have been adopted (13). In all cases, the appropriate equation of the general form

$$C = \sum_{i=1}^{n} C_i(\lambda_i\tau)^{-1}(e^{-\lambda_i t'} - e^{-\lambda_i t})$$

was fit to observed time courses of plasma MB concentration by weighted nonlinear least-squares regression using PCNONLIN (Statistical Consultants, Lexington, Ky.). The equation was derived according to the principles developed by Benet and simplified to eliminate compartmental attributes (1). Thus, the value of t' is zero until the infusion of duration τ has terminated, upon which it becomes defined as $t' = t - \tau$, where t denotes time from the initiation of treatment. The coefficients C_i are intercept values, corresponding to i.v. bolus administration of the dose, of each log-linear phase with a slope $-\lambda_i$, such that $\lambda_1 > \lambda_2 > ... > \lambda_z$. Parameters corresponding to the terminal decay phase are designated with a subscript z by convention. Each plasma profile was subject to repeated regression analyses whereby the number of exponential terms in the fitted equation and influence of the weighting factor, y_{obs}^{-n} ($0 \le n \le 2$), were both evaluated to identify the simplest equation that best described the data on the basis of the statistical output generated by the program, particularly the residual analysis, the weighted sum of the squared residuals, and the correlation matrix of the parameter estimates. With few exceptions, weighting factors of $1/y_{obs}^2$ were found to be optimal. Parameter values of the best-fit equation were used to calculate all pharmacokinetic terms. Renal clearance (CL_R) was calculated according to the equation $CL_R = A_{e(0\to t_n)}/AUC_{(0\to t_n)}$, where $A_{e(0\to t_n)}$ is the cumulative amount of drug excreted unchanged in the urine from the start of dosing to the end of the last void interval and $AUC_{(0\to t_n)}$ represents the area under the plasma profile of the drug during this period (15). Values of $AUC_{(0\to t_n)}$ were calculated nonlinear regression estimates of the macroscopic disposition parameters C_i and λ_i.

Mean values of pharmacokinetic terms are reported as the arithmetic means ± SD, with the exception of half-lives ($t_{1/2,i}$) and MRT, for which the harmonic means and jacknife estimates of the SD were calculated (10). In consideration of the small sample sizes ($n=3$) and theoretical non-Gaussian population distribution of the $t_{1/2,i}$ and MRT values, differences in pharmacokinetic parameters between species were evaluated statistically by using the nonparametric Mann-Whitney U test (5). A level of $P < 0.05$ was considered significant.

RESULTS

Murine pharmacokinetics. The disposition of MB in mice treated by rapid i.v. injection with doses of 0.98, 4.74, and 9.49 mg/kg of body weight was characterized. These doses were well tolerated, with no observable indications of toxicity, whereas 25 mg of MB per kg proved to be lethal in a matter of minutes. Maximum drug plasma levels achieved at the end of the injection [$C(\tau)$], increased from 21.3 to 143.4 μg/ml as the dose was escalated. As shown in Fig. 2, the plasma concentration-time profiles of MB in mice were most satisfactorily described by triexponential equations. Initial loss of the drug proceeded rapidly, as its concentration decreased by approximately one log decade within 60 min after dosing. The half-lives of the two initial disposition phases afforded mean values (± SD) of 4.5 ± 2.5 and 27.4 ± 23.9 min, respectively (Table 1). Nevertheless, because of the substantial contribution (44.6% ± 12.9%) of the terminal phase to the area under the plasma profile from time zero to infinity (AUC) the magnitude of the MRT (2.07 ± 0.31 h) approached that of the biological half-life, $t_{1/2,z}$, 2.79 ± 0.82 h. Within this dose range, the apparent total plasma clearance (CL) of MB exhibited a mean value of 2.44 ml/min/kg with a 22.1% coefficient of variation. Values of the apparent volumes of distribution ranged from 0.053 ± 0.009 liters/kg for the central compartment (V_1) to 0.60 ± 0.08 liters/kg for the total body apparent volume of distribution (V_z).

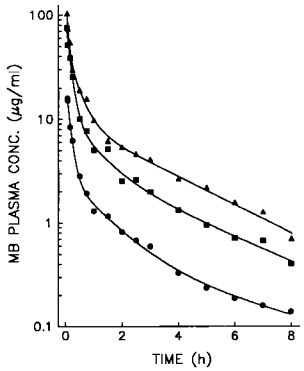

FIG. 2. Plasma concentration-time profiles of MB in mice treated by 1-min i.v. injection with doses of 0.98 (●), 4.72 (■), and 9.49 (▲) mg/kg. Experimental points are the geometric means of observed plasma levels in three mice per time point. The solid lines represent the best-fit curves of the experimental data (points) as determined by nonlinear regression. conc., concentration.

Toxicity in dogs. There were no symptomatic or significant pathological indications of drug-related toxicity among the dogs treated with 0.88 to 8.63 mg of MB per kg of body weight by 15-min i.v. infusion. Similarly, a total dose of 97.0 mg/kg given as a 72-h continuous i.v. infusion was well tolerated. However, during the short infusion of a 24.0-mg/kg dose, the dog began to display behavioral changes that were clearly indicative of an adverse reaction to the drug. Immediately upon termination of the infusion, the dog vomited, then gradually lapsed into a state of complete unconsciousness for approximately 2 h. During this period, physical examination revealed the following: very dark mucous membranes, dilated and palpebral pupils, diminished respiration (11 per min), and a complete loss of righting reflex. Recovery was indicated by the development of moderate shivering with gradual improvements in respiration and restoration of reflexes until the dog appeared normal at 10 h after dosing. While the underlying physiological effects cannot be identified without histopathological evidence, these observations indicate that the animal

TABLE 1. Pharmacokinetic and derived parameters of MB in mice

Parameter[a] (units)	Observed values with dose (mg/kg) of:			Mean ± SD
	0.98	4.74	9.49	
$C(\tau)$ (μg/ml)	21.3	83.5	143.4	
C_1 (μg/ml)	19.6	76.0	107.5	
C_2 (μg/ml)	2.36	7.93	38.8	
C_z (μg/ml)	0.47	3.66	9.72	
$t_{1/2,1}$ (min)	5.9	6.3	2.9	4.5 ± 2.5
$t_{1/2,2}$ (min)	53.5	37.1	15.7	27.4 ± 23.9
$t_{1/2,z}$ (h)	4.20	2.58	2.22	2.79 ± 0.82
MRT (h)	2.49	1.83	1.99	2.07 ± 0.31
CL (ml/min/kg)	1.89	2.46	2.97	2.44 ± 0.54
V_1 (liters/kg)	0.044	0.054	0.061	0.053 ± 0.009
V_{ss} (liters/kg)	0.28	0.27	0.35	0.31 ± 0.04
V_z (liters/kg)	0.69	0.55	0.57	0.60 ± 0.08
AUC (μg · h/ml)	8.64	32.2	53.2	
AUC_1 (%)	31.9	35.5	14.0	27.1 ± 11.5
AUC_2 (%)	35.2	22.0	27.5	28.2 ± 6.6
AUC_z (%)	32.9	42.5	58.5	44.6 ± 12.9

[a] Abbreviations of pharmacokinetic terms: $C(\tau)$, plasma concentration of drug at the end of infusion; AUC_i, contribution of the ith disposition phase to AUC.

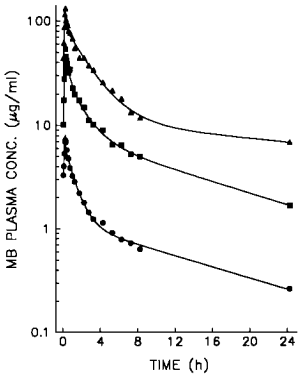

FIG. 3. Plasma concentration-time profiles of MB in female dogs treated by 15-min i.v. infusion with doses of 0.88 (●), 4.95 (■), and 24.0 (▲) mg/kg. The experimental data (points) and best-fit curves (solid lines) generated by nonlinear regression analysis are shown. conc., concentration.

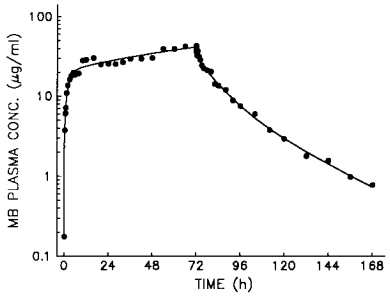

FIG. 4. Time course of MB in a female dog during and after administration of a total dose of 97.0 mg/kg by 72-h continuous i.v. infusion. The experimental data (points) are shown together with the best-fit curve (solid line) determined by nonlinear regression. conc., concentration.

was close to complete cardiac collapse and also suggest a possible neurological component in the toxic manifestations of the drug.

Plasma pharmacokinetics in dogs. Plasma concentration-time profiles depicting the experimentally determined plasma levels and best-fit curves of MB during and after administration to dogs by 15-min i.v. infusion are shown in Fig. 3. In each case, nonlinear regression analysis revealed that the plasma MB concentration decreased triexponentially. Comparative analysis of the pharmacokinetic parameters and derived terms for the individual dogs, which are summarized in Table 2, suggested that the disposition of MB was linear at the nontoxic doses of 0.88 to 8.63 mg/kg. However, there appeared to be an alteration in pharmacokinetic behavior with the 24.0-mg/kg dose, which was extremely toxic, as noted above. Specifically, whereas the peak plasma MB concentration was notably lower than predicted, the values of $t_{1/2,z}$, MRT, volume of distribution at steady-state (V_{ss}), and V_z were considerably higher than those observed for the dogs treated with the three lower doses. The prolongation of the terminal phase of MB in the dog that received the toxic dose is readily apparent in Fig. 3. Therefore, the mean values of the pharmacokinetic parameters (Table 2) were calculated with the exclusion of those corresponding to the 24.0-mg/kg dose of MB.

TABLE 2. Pharmacokinetic and derived parameters of MB in dogs

Parameter[a] (units)	Observed values with dose (mg/kg) of:				Mean ± SD[b]
	0.88	4.95	8.63	24.0	
$C(\tau)$ (μg/ml)	8.2	48.2	68.8	133.6	
C_1 (μg/ml)	13.7	26.0	40.2	167.7	
C_2 (μg/ml)	5.48	22.6	34.7	79.4	
C_z (μg/ml)	1.15	7.97	13.2	11.3	
$t_{1/2,1}$ (min)	1.7	16.3	8.4	3.0	3.9 ± 9.4
$t_{1/2,2}$ (min)	43.8	73.3	82.4	101.1	61.7 ± 25.0
$t_{1/2,z}$ (h)	11.2	10.7	13.2	33.0	11.6 ± 1.2
MRT (h)	12.2	11.4	15.0	35.1	12.3 ± 1.8
CL (ml/min/kg)	0.59	0.48	0.44	0.54	0.50 ± 0.08
CL_R (ml/min/kg)	0.045	0.053	0.021	0.020	0.040 ± 0.017
CL_R/CL (%)	7.6	11.1	4.7	3.7	7.8 ± 3.2
$f_{e,ur}$(24 h) (% of dose)	6.8	9.5	3.7	2.1	6.7 ± 2.9
V_1 (liters/kg)	0.043	0.087	0.098	0.093	0.076 ± 0.029
V_{ss} (liters/kg)	0.43	0.33	0.40	1.13	0.39 ± 0.05
V_z (liters/kg)	0.57	0.44	0.50	1.54	0.50 ± 0.07
AUC (μg · h/ml)	24.8	173.0	328.3	744.6	
AUC_1 (%)	2.2	5.9	2.5	1.6	3.5 ± 2.1
AUC_2 (%)	23.3	23.0	20.9	25.9	22.4 ± 1.3
AUC_z (%)	74.5	71.1	76.6	72.4	74.1 ± 2.8

[a] $f_{e,ur}$(24 h), fraction of the dose excreted in the urine between 0 and 24 h after dosing. Other abbreviations are defined in Table 1, footnote *a*, and in the text.
[b] Parameters for the 24.0-mg/kg dose were not included in the calculation of mean parameters because of drug toxicity.

The peak plasma drug concentration achieved at the end of the infusion ranged from 8.2 μg/ml at the starting dose of 0.88 mg/kg to 68.8 μg/ml in the dog treated with 8.63 mg/kg. The $t_{1/2,1}$ was very rapid and variable, ranging from 1.7 to 8.4 min in individual dogs about a mean of 3.9 ± 9.4 min. However, as the $t_{1/2,2}$ (61.7 ± 25.0 min) was more than 15 times greater than $t_{1/2,1}$ plasma MB levels declined only by a factor of 10 during the 6 to 8 h preceding the terminal phase. Thus, with an average contribution of 74.1% ± 2.8% to the AUC, the terminal exponential phase was the primary determinant of drug disposition. Consistent with the hydrophilic character of MB, which is undoubtedly charged at physiologic pH, its mean V_{SS} in the dogs, 0.39 ± 0.05 liters/kg, was roughly equivalent to two-thirds the volume of total body water. The values of the pharmacokinetic terms associated with drug elimination were very consistent in the three studies. Coefficients of variation for the mean values of $t_{1/2,z}$ (11.6 h) and MRT (12.3 h) were 10.3 and 14.6%, respectively. Thus, as implied by the magnitudes of these parameters, MB was cleared rather slowly from the dogs (CL, 0.50 ± 0.08 ml/min/kg). Urinary excretion represented a relatively minor route of elimination, as the cumulative amount of unchanged drug found in the urine during 24 h accounted for only 3.7 to 9.5% of the dose, providing a mean value of 0.04 ± 0.017 ml/min/kg for the CL_R of MB for the three dogs.

The mean CL for the dogs treated with doses of 0.88 to 8.63 mg/kg was employed to calculate an infusion rate to maintain levels in plasma within a range of one to two times the 50% effective concentration of MB against HIV in vitro (18 μg/ml) for a period of 3 days (11). Simulations indicated that a total dose of 90 mg/kg administered as a 72-h continuous i.v. infusion would provide a concentration in plasma of 20 μg/ml at 10 h and 34 μg/ml at the end of the infusion. In the actual experiment, a single female dog received a total dose of 97.0 mg/kg, as indicated by HPLC analysis of the drug concentration in the formulation administered. As shown in Fig. 4, plasma MB levels exceeded 20 μg/ml near 8 h and continually increased throughout the duration of the infusion to a maximum concentration of 41.4 μg/ml. This was slightly lower than the theoretical steady-state concentration of 46.6 μg/ml calculated from the actual rate of infusion and observed CL (0.48 ml/min/

kg). Following the infusion, the loss of drug from plasma proceeded in a triexponential manner. Whereas the CL and CL_R (0.033 ml/min/kg) were comparable to the values afforded by the short infusion protocol, the magnitudes of the apparent $t_{1/2,z}$ (25.6 h), MRT (26.5 h), V_{ss} (0.77 liters/kg), and V_z (1.07 liter/kg) were all significantly greater. Other observed parameters included the fraction of the dose excreted in the urine between time zero and 168 h after dosing (5.5%) and an AUC of 3,351.5 μg · h/ml. The considerable prolongation of the apparent $t_{1/2,z}$ was undoubtedly responsible for the failure to achieve the onset of steady-state within 48 h after starting the infusion, as predicted by the short infusion studies ($t_{1/2,z}$, 11.6 h).

DISCUSSION

The administration of nontoxic doses of MB (5 to 9 mg/kg) to mice and dogs by short i.v. infusion provided peak levels in plasma within the 50- to 100-μg/ml range required to completely inhibit the cytopathic effects of HIV-1 and HIV-2 upon cultured human lymphoblastoid cells in various antiviral assays (11). At these doses, the drug exhibited apparent first-order kinetics, affording distinctly triexponential plasma concentration-time profiles in mice as well as dogs. However, prominent interspecies differences in the disposition of the drug were evident. Concentrations in plasma achieved at the end of the 1-min dosing interval in mice were more than twice as high as those observed in dogs treated with similar doses but infused over a period of 15 min. Nevertheless, plasma drug levels in mice decreased nearly 200-fold during the subsequent 8 h, whereas the concentration of drug in the dogs declined by only a factor of 10 during this same time interval. Consequently, while the plasma MB concentration decayed below 18 μg/ml, its 50% effective concentration against HIV in vitro (11), within 30 min after the mice received a dose of 9.49 mg/kg, potentially therapeutic drug levels were maintained in a dog for 3 h after treatment with a similar dose (8.63 mg/kg).

Consistent with these observations, the pharmacokinetic parameters associated with drug elimination are statistically different in mice and dogs. The mean $t_{1/2,z}$ (11.6 ± 1.2 h) and MRT (12.3 ± 1.8 h) of MB in dogs were, respectively, four and six times longer than those observed for mice. Similarly, the mean CL for dogs (0.50 ± 0.08 ml/min/kg) was fivefold lower than the rate for mice. However, there are no statistical differences in the respective mean values of V_1, V_{ss}, or V_z between the two species, indicative of similar binding characteristics of the drug with plasma proteins in relation to tissues. This suggests that the observed differences in drug elimination are attributable to marked interspecies differences in its intrinsic clearance.

There is no evidence of dose-dependent disposition in dogs treated by 15-min i.v. infusion with 0.88 to 8.63 mg of MB per kg. Nevertheless, when 24.0 mg of the drug per kg was similarly administered, the $t_{1/2,z}$, MRT, V_{ss}, and V_z were all substantially higher than their mean values at the lower doses. These effects may be associated with the pronounced toxicity observed at this dose level. However, seemingly analogous alterations in pharmacokinetic behavior were evident upon treatment of a dog with a total dose of 97 mg/kg by 72-h continuous i.v. infusion, which was well tolerated. In spite of the changes in the aforementioned pharmacokinetic parameters, the AUC increased in proportion to the administered dose ($r = 0.999$; $n = 5$). Thus, there are no indications of dose- or schedule-dependent changes in the apparent CL of MB. In the 15-min infusion studies, pharmacokinetic sampling was terminated 24 h after dosing, whereas specimens were obtained for 4 days after the 72 h infusion. This facilitated a marked improvement in the characterization of the postinfusion region of the plasma profile. Computer simulations (data not shown) revealed that the time courses of MB in plasma during the initial 24 h after dosing on either schedule were essentially superimposable. Thereafter, the rate of drug loss in the best-fit curve of the 72-h infusion study became progressively lower than the rate for the corresponding extrapolated region of the plasma profile generated from the mean pharmacokinetic parameters for dogs treated by 15-min i.v. infusion. Thus, the possibility that terminating sampling at 24 h resulted in an underestimate of the apparent $t_{1/2,z}$ was suggested. Regardless, the AUC for the profile generated from the mean parameters for the 15-min infusion studies was only 9.1% less than postinfusion AUC for the 72-h study, thereby providing indistinguishable values for CL within the confines of intersubject variability.

The question pertaining to the true $t_{1/2,z}$ of MB could be resolved by repeating the short infusion studies in dogs, with an appropriate sampling schedule to definitively characterize the terminal disposition phase. However, since in vitro studies suggest that effective anti-HIV therapy will require continuous exposure to the compound (11), it will be administered to a cohort of dogs by continuous i.v. infusion in the ensuing definitive toxicological evaluation of the clinical formulation. The acquisition of pharmacokinetic information will be an integral component of these studies, thereby providing an opportunity to reevaluate the pharmacokinetic behavior of the drug. This approach was considered preferable given current limitations on the supply of this naturally occurring agent.

The major pathways by which MB is eliminated from the body have not been identified. On average, only 6.8% ± 2.9% ($n = 5$) of the dose was excreted as unchanged drug in the urine of all dogs treated with MB. The structural characteristics of MB suggest that biliary excretion and metabolism could both be prominent routes of elimination. While the high molecular weight and polarity should facilitate biliary excretion of the unchanged drug, the presence of multiple phenolic groups represent potential sites for conjugative as well as oxidative biotransformations. However, peaks consistent with drug metabolites were not observed in the chromatograms of plasma or urine specimens, undoubtedly because of the highly specific nature of the analytical method, which employs fluorescence detection to monitor the elution of components upon reversed-phase HPLC. Biotransformation by either phase I or phase II pathways would very likely alter the physicochemical properties of the drug sufficiently to preclude detection under the assay conditions used to monitor the parent drug.

In these studies, we have demonstrated that the concentrations of MB effective against HIV-1 and HIV-2 in vitro can be achieved systemically in preclinical animal models after i.v. administration without incidence of toxicity. As previously indicated, extravascular administration of MB does not appear to be clinically feasible because of its poor oral bioavailability and low therapeutic index in vitro. While good oral bioavailability is indisputably advantageous, a requisite for i.v. administration should not, in itself, preclude the clinical evaluation of investigational agents for the treatment of AIDS. This is particularly relevant to new anti-HIV therapies, since the few currently approved drugs have a documented inability to completely arrest the progression of AIDS, and thus, a critical need for new treatment remains (9). In fact, the advantages of employing an i.v. infusion dosing regimen for phase I clinical trials with investigational chemotherapeutic agents which, by their very nature, have the potential to elicit serious toxicities are commonly recognized. Given the limited ability of animal models to accurately predict pharmacokinetic behavior and

maximum tolerated doses in humans (4, 6, 8, 12), an infusion protocol facilitates the immediate termination of drug input in the event of toxicity during dose administration. This may be extremely difficult, at best, following extravascular administration. In the case of MB, a continuous i.v. infusion protocol may be preferable to insure the maintenance of potentially therapeutic drug levels, insofar as subeffective concentrations apparently stimulate in vitro viral replication (11). Efforts to identify alternative routes of administration and explore structural modifications designed to promote oral absorption or enhance potency would certainly be justified if MB exhibits encouraging clinical efficacy.

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