Simultaneous Modeling of the Pharmacokinetics and Methemoglobin Pharmacodynamics of an 8-Aminoquinoline Candidate Antimalarial (WR 238605)

Ralf P. Brueckner[1,2] and Lawrence Fleckenstein[1]

Received December 12, 1990; accepted June 3, 1991

Methemoglobin (MHb) formation can be a clinically significant and dose-limiting side effect of 8-aminoquinoline antimalarials. MHb may also protect against cyanide poisoning. A two-compartment pharmacokinetic model, linked to a sigmoid E_{max} pharmacodynamic model, was developed to predict the MHb levels after administration of 8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)phenoxy] quinoline succinate (WR 238605 succinate), a primaquine analogue. Six healthy male beagle dogs received four daily doses of 6.0 mg/kg (base) orally. Forty plasma drug concentrations and 19 MHb levels (effect) were determined over 7 weeks on each dog. Compartmental and noncompartmental pharmacokinetic and parametric and nonparametric pharmacodynamic analyses were performed. Model parameters (mean ± SD) included a V_{ss}/f of 18.5 ± 2.8 L/kg, CL/f of 83 ± 24 ml/hr/kg, terminal elimination $t_{1/2}$ of 169.7 ± 52.0 hr, $t_{1/2}k_{eo}$ of 123.0 ± 22.4 hr, an E_{max} of 31.3 ± 15.9% MHb, an EC_{50} of 596 ± 128 ng/ml, and a sigmoidicity coefficient (n) of 1.94 ± 0.47. The model was then validated in three additional dogs given three different dosing regimens. It predicted the peak plasma concentrations and MHb levels and the times of their occurrence well. This model could be useful for dose and sampling time selection in further animal studies and initial human phase I clinical testing.

KEY WORDS: 8-aminoquinoline; antimalarial; cyanide poisoning; methemoglobin; pharmacokinetic-pharmacodynamic model.

INTRODUCTION

Malaria is one of the most common infectious diseases of man. Approximately 2.4 billion people live in malaria-endemic areas. This is about half the world's population. Each year 100–300 million people are infected with malaria, and of these, 1–2.5 million die (1).

Primaquine is the drug of choice for the treatment of the liver stage of *Plasmodium vivax* and *Plasmodium ovale* malaria (2). However, primaquine resistant malaria is becoming a more significant medical problem (3). Primaquine is also associated with significant toxicities, including hemolysis, leukopenia, and arrhythmias (4). This drug, as well as other 8-aminoquinoline antimalarials, can also produce high, dose-related levels of methemoglobin (MHb), which can be a clinically significant and dose-limiting side-effect.

High MHb levels not only are a common side effect of many 8-aminoquinoline antimalarials, but also may be the intended therapeutic effect of one class of drugs used to treat cyanide poisoning or protect against cyanide exposure. It has been demonstrated in dogs that drugs which produce MHb levels of 10–15% can effectively protect against several LD_{50}'s of cyanide (5).

The Walter Reed Army Institute of Research is developing a new primaquine analogue, 8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)-phenoxy] quinoline (WR 238605) succinate, as a possible replacement for primaquine and as a drug that may protect against cyanide poisoning. The Beagle is considered an appropriate animal model for predicting MHb forming and clearing activity in humans (5–7). The purpose of this study was to develop a combined pharmacokinetic-pharmacodynamic (PK-PD) model for WR 238605 plasma concentrations (C_p) and MHb levels in Beagles. This model could then guide further studies with this drug in animals and man. A two-compartment PK model, linked to a sigmoid E_{max} PD model, was developed in a study group of dogs and subsequently validated in an additional test group.

MATERIALS AND METHODS

Drug

The drug, 8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)phenoxy] quinoline (WR 238605) succinate, was synthesized by Ash Stevens, Inc. (Detroit, MI). Bottle number BK73252 was used, which has a purity of 99.9 ± 0.5% (8). The drug was prepared as a 100 mg/ml suspension in 1% (w/v) methylcellulose (4000 cP, lot No. 776402, Fisher Scientific Co., Fair Lawn, NJ) and 0.4% (v/v) Tween 80 (lot No. 90F-0462, Sigma Chemical Co., St. Louis, MO). The suspension was kept refrigerated in an amber bottle after preparation and during the dosing period.

Animals

Six healthy male Beagles, age 10–21 months and weighing 9.1–14.5 kg, were acquired from Laboratory Research Enterprises, Inc. (Kalamazoo, MI). These dogs had not been used in previous studies. They were certified healthy by our institution's veterinarian and had normal complete blood counts, chemistry, and liver function tests. Baseline MHb determinations were done weekly for 2 months and found to be normal and stable (<2%). The study was approved by our Laboratory Animal Care and Use Committee. The dogs were cared for in accordance with the principles in the *Guide for the Care and Use of Laboratory Animals* (Department of Health, Education and Welfare Publication, NIH 85-23). They were housed in individual runs measuring 4 × 10 ft. The environment was controlled within a temperature range of 68–72°F and a humidity range of 40–60%. They were provided a measured amount of Purina dog chow each day and water ad libitum.

Dosing

Each dog received four daily oral doses of 6.0 mg/kg of

[1] Department of Pharmacology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

[2] To whom correspondence should be addressed.

0724-8741/91/1200-1505$06.50/0

WR 238605 base (7.5 mg/kg succinate), on study days 0–3 (hr 0, 24, 48, 72). The animals' weight on day −3 was used for all four doses. The animals were all dosed within a 17-min period in the mornings. They were fasted for 12 hr before and 4 hr after dosing. At the time of each dose, the appropriate amount of well-stirred suspension was pipetted into a size 000 gelatin capsule (Parke-Davis, Morris Plains, NJ) and immediately administered to the animal.

Sampling

Blood samples were obtained for determination of plasma drug concentrations and methemoglobin levels over 7 weeks. Samples for drug concentration determinations were drawn at 0, 2, 4, 8, 12, 16, 20, 24, 26, 28, 32, 36, 48, 50, 52, 56, 60, 72, 74, 76, 80, 84, 88, 92, 96, 100, 104, 120, 144, 168, 192, 216, 240, 264, 336, 432, 600, 768, 936, and 1104 hr. Samples were collected from the anterior cephalic or jugular vein using a 21-G, 1.25-in. needle and syringe and transferred to VACUTAINER tubes containing EDTA. The specimens were immediately centrifuged and frozen at −70°C until assayed. A study had previously demonstrated stability of drug in plasma over the storage time at this temperature (9).

At 0, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 336, 432, 600, 768, 936, and 1104 hr, additional blood samples were collected for MHb determination. They were kept on ice after venipuncture and analyses were begun within 30 min. Three milliliters of blood was drawn for each drug level, and 2 ml for each MHb determination.

Analytical

Plasma WR 238605 concentrations were determined by HPLC (9). The method used a Waters Intelligent Sample Processor (Model 710B, Waters Associates, Milford, MA), a Beckman Model 110B solvent delivery module, and an Altex Ultrasphere silica gel column (5-μm particle size, 4.6 × 250 mm, Beckman Instruments Inc., Berkeley, CA), an aqueous mobile phase, a Hewlett-Packard Reporting Integrator No. 3392A (Hewlett-Packard Co., Santa Clara, CA), and a Perkin-Elmer 204-A fluorescence spectrophotometer detector with an excitation wavelength of 375 nm and an emission wavelength of 480 nm (Perkin-Elmer Corp., Norwalk, CT). Briefly, 10 μl of internal standard [WR 6026; 6-methoxy-8-(6-diethylaminohexylamino lepidine dihydrochloride) solution (104 μg/ml)] and 0.1 ml of 0.1 *N* NaOH buffer were added to 0.2 ml of the plasma sample. Then 3 ml of methyl-t-butyl ether extracting solvent was added, mixed, and centrifuged for 10 min at 3000*g*. The organic layer was then evaporated to dryness under nitrogen. The residue was reconstituted with 200 μl (or for samples with suspected drug concentrations greater than 150 ng/ml, 400 μl) of acetonitrile/water (1:1, v/v). This was transferred to a autoinjector insert and injected into the column with a flow rate of 1.0 ml/min. All solvents were HPLC grade; all chemicals were reagent grade.

Total hemoglobin (Hb) was determined using the commercially available Unopett assay system (Becton-Dickinson Co., Rutherford, NJ) and Perkin-Elmer Lambda 3B UV/VIS spectrophotometer (Oak Brook, IL). This method converts all Hb in blood (except sulfhemoglobin) to cyan-MHb using a mixture of potassium ferricyanide and potassium cyanide. The absorbance at 540 nm is compared to a standard curve prepared daily using hematology controls (Hematall-C, Fisher Scientific, Orangeburg, NY).

MHb levels were measured by the method of Dubowski (10) as modified by Anders and Chung (11). This spectrophotometric method is based upon the characteristic disappearance of the absorption of MHb at 635 nm after the addition of KCN. Briefly, 0.2 ml of whole blood is diluted in 2 ml of 0.25 *M* sodium phosphate buffer, pH 7.5. The red cells are lysed with 10 mg of Triton X-100 (1 drop using a 21-G needle) and vortexed until clear. The hemolysate is centrifuged at 1200*g* for 15 min, and the supernatant again centrifuged. One milliliter of this supernatant is placed into a semimicro cuvette (path length = 1 cm), and the absorbance A_1 read against blank buffer at 635 nm. Then 50 μl of 614 m*M* (2 mg) KCN is added, converting all the MHb to cyan-MHb and the absorbance A_2 read. The amount of MHb is calculated as

$$\text{MHb(g\%)} = \frac{A_1 - A_2}{1.89} \times \text{DF} \quad (1)$$

where DF, the dilution factor, is 11. MHb results are expressed as percentage of total hemoglobin.

Data Analysis

A noncompartmental analysis was performed on the time–concentration data. The area under the curve (AUC) was calculated by the linear trapezoidal method until t_{last} and extrapolated to infinity using the slope of the terminal elimination phase (λ_z). The area under the moment curve (AUMC) was similarly calculated. Clearance (CL/*f*) was then calculated as

$$\text{CL}/f = \frac{\text{dose}}{\text{AUC}} \quad (2)$$

mean residence time (MRT), corrected for absorption, as

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}} - \frac{1}{k_a} \quad (3)$$

and volume of distribution at steady state (V_{ss}/f) as

$$V_{ss}/f = \text{CL} \times \text{MRT} \quad (4)$$

A nonparametric PD analysis of the concentration–MHb data was performed (12) to guide selection of the appropriate PD model and to obtain initial estimates of k_{eo}. This first-order equilibration rate constant describes the rate of loss of drug from the effect site and regulates the temporal delay between plasma drug concentration and effect. Extended nonlinear least-squares regression was then used to fit the combined PK-PD model to the WR 238605 plasma concentration and MHb data. A one- and two-compartment open model (1- and 2-COM) with first-order absorption and elimination, linked to an effect compartment (13), was written using MKMODEL (Elsevier/Biosoft, Cambridge, U.K.). For the plasma concentration, the 1-COM was parameterized as

$$C(t) = \frac{\text{dose}}{V} \times \frac{k_a}{k_a - \frac{CL}{V}} \times (e^{-\frac{CL}{V} \times t} - e^{-k_e \times t}) \quad (5)$$

For the effect site concentration, the link model was parameterized as

$$C_e(t) = \frac{\text{dose} \times k_a \times k_{eo}}{V} \times \left[\frac{e^{-\frac{CL}{V} \times t}}{\left(k_a - \frac{CL}{V}\right) \times \left(k_{eo} - \frac{CL}{V}\right)} + \frac{e^{-k_a \times t}}{\left(\frac{CL}{V} - k_a\right) \times (k_{eo} - k_a)} + \frac{e^{-k_{eo} \times t}}{\left(\frac{CL}{V} - k_{eo}\right) \times (k_a - k_{eo})} \right] \quad (6)$$

The 2-COM was similarly parameterized. This effect-site concentration (C_e) was used in the sigmoid E_{max} PD model to describe the effect E (%MHb):

$$E = E_o + \frac{E_{max} \times C_e^n}{EC_{50}^n + C_e^n} \quad (7)$$

For individual animals, the choice of the appropriate PK model (1- or 2-COM) was based upon standard goodness-of-fit criteria. These include the visual inspection of the observed and predicted concentration and effect curves and residual plots, comparison of the standard errors and confidence intervals of the parameter estimates, and values of the log-likelihood and Schwarz criteria. Incorporating a lag time did not improve the fits, so a lag time was not used in the final model.

Model Validation

The mean parameter values for the four animals that were best fit by a 2-COM were chosen for the predictive model of WR 238605 plasma concentrations and %MHb levels. Predicted C_p and %MHb were compared with those measured in three additional dogs, given three dosing regimens that were different from the regimen used to develop the model. The animals in this test group received doses totaling 9, 27, and 25 mg/kg (3.0 mg/kg × 3 daily doses, 18.0 mg/kg loading dose followed by 3.0 mg/kg × 3 daily doses, and 10.0 mg/kg loading dose followed by 5.0 mg/kg × 3 daily doses).

RESULTS

The animals tolerated the drug well. One animal (9A04) vomited once 30 min after administration of the fourth dose, and only a small amount of drug was noted in the emesis. All but one dog had some loose stools, occurring 2–4 days after the last dose. The only other clinical findings were a cyanotic appearance of the tongue and sclera, when MHb levels were greater than about 10%. The total hemoglobin levels fell by an average of 1.76 ± 0.82 g/dl on day 10, representing a 10% average decrease from baseline. They returned to baseline approximately on day 22.

The WR 238605 plasma concentrations in two of the dogs (9A01 and 9B07) were best described by the 1-COM, using the goodness-of-fit criteria described under Data Analysis. Those in the other four dogs (9A03–9A06) were best described by a 2-COM. The parameters AUC, V_{ss}/f, CL/f, and MRT, obtained from the compartmental PK analysis, were within 10% of those obtained from noncompartmental PK analysis (Table I). WR 238605 had a highly variable absorption half-life of 2.9 ± 1.9 hr (mean ± standard deviation), a long elimination half-life (169.7 ± 52.0 hr), long mean residence time (MRT = 269 ± 85 hr), and large volume of distribution (V_{ss}/f = 18.5 ± 2.8 L/kg) (Table II). Clearance was estimated to be 83 ± 24 ml/hr/kg. The nonparametric PD analysis suggested a sigmoid E_{max} model. The observed peak %MHb averaged 14% (range, 7.6–21.3%) and the time to peak %MHb averaged 196 hr (range, 168 to 240 hr). The $t_{1/2}k_{eo}$ had a mean value of 123.0 ± 22.4 hr. E_{max} was 31.3 ± 15.9% MHb, EC_{50} was 596 ± 128 ng/ml, and sigmoidicity coefficient (n) was estimated to be 1.94 ± 0.47 (Table III). Harmonic means ± jackknife standard deviations are reported for the half-lives and the clearance derived from the noncompartmental calculation (14).

The standard errors and confidence limits of the param-

Table I. Comparison of Noncompartmental (Noncomp) and Compartmental (Comp) Pharmacokinetic Parameters

	AUC (ng · hr/ml)		V_{ss}/f (L/kg)		CL/f (ml/hr/kg)		MRT (hr)	
Dog ID	Noncomp	Comp	Noncomp	Comp	Noncomp	Comp[a]	Noncomp	Comp
9A01	290,512	289,363	20.9	17.9	83	82	253	218
9A03	208,800	200,588	22.7	20.2	115	119	198	169
9A04	271,920	263,632	16.3	13.3	88	91	185	146
9A05	488,256	479,777	19.7	20.1	49	50	401	402
9A06	266,864	261,377	21.3	18.1	90	92	237	197
9B07	374,388	371,841	22.1	21.2	64	64	344	329
Mean	316,790	311,097	20.5	18.5	76[b]	83	269	244
SD	99,576	99,551	2.3	2.8	25	24	85	100
%CV	31	32	11	15	34	29	32	41

[a] Standard error of each clearance estimate range (30.30–45.40).
[b] Harmonic mean and jackknife standard deviation (14).

Table II. Compartmental Pharmacokinetic Analysis[a]

Dog ID	V_1 (L/kg)	$T_{1/2}\ k_a$ (hr)	$T_{1/2}\ \lambda_1$ (hr)	$T_{1/2}\ \lambda_z$ (hr)
9A01	—	1.6	—	150.8
9A03	8.1	2.6	16.6	155.4
9A04	3.7	6.8	5.6	119.5
9A05	10.5	2.5	26.4	308.8
9A06	5.0	4.9	6.8	158.6
9B07	—	0.9	—	228.0
Mean	6.8	2.1[b]	9.4[b]	169.7[b]
SD	3.1	1.9	6.4	52.0
%CV	45	89	68	31
SEE range[c]	0.61–8.69	0.37–15.39	3.46–12.16	4.34–20.18

[a] Dogs 9A01 and 9B07 fit to a one-compartment model; dogs 9A03–9A06 fit to a two-compartment model.
[b] Harmonic mean and jackknife standard deviation.
[c] Standard errors of each parameter estimate.

eter estimates in the individual dogs were greatest for k_a, λ_1, V_1, and E_{max}. Examination of the data in each dog showed marked variation about the expected peak plasma concentration from one dose to the next (Fig. 1, inset).

There was a long delay between the rise and the fall of plasma WR 238605 concentrations and effect (Fig. 1). When %MHb was plotted against plasma concentration, a large, counterclockwise hysteresis loop was observed (Fig. 2A). After the combined PK-PD model collapsed the hysteresis loop, the sigmoid E_{max} concentration–effect relationship was apparent (Fig. 2B).

The predictive model used the PK and PD parameters of the four dogs that were best fit to a 2-COM. It provided good estimates of plasma WR 238605 concentrations and MHb levels and the times of their occurrences in the three additional dogs of the validation group (Fig. 3). The initial plasma concentrations in the dogs receiving the loading doses were overpredicted by the model (Figs. 3B and C). This could be a result of incomplete bioavailability at these high doses. The differences in the predicted and observed peak %MHb values were less than 2% MHb for two of the animals (Figs. 3A and B), but this difference was 7.8% MHb in the third animal (Fig. 3C).

DISCUSSION

This is the first combined PK-PD model of the candidate antimalarial WR 238605. The model describes the plasma WR 238605 concentrations and MHb levels in individual animals in the study group. Further, this model was subsequently validated in an additional group of animals receiving dosing regimens not used for the model development. In this validation group, the model predicts the plasma concentrations, MHb levels, and times of their occurrence. This provides greater confidence that the model could be of use in designing subsequent animal toxicity studies and initial human clinical studies. The model may provide insight into the effects of different dosing regimens and may be useful in selecting appropriate sampling times.

A single-dose study is obviously simpler to perform and is mathematically less complicated than a multiple-dose regimen. However, based on a toxicity study (15), it appeared that the large (25-mg/kg) single dose that would have been required to produce the target MHb level of 10–15% would not have been completely absorbed. Also, larger doses were associated with vomiting and diarrhea. Gastrointestinal disturbances such as these may compromise the estimates of the PK parameters. For these reasons, a dosing regimen of four daily doses was chosen for this study. The animals in our study did not have any gastrointestinal problems that were thought significant enough to affect the estimates of the PK parameters.

The C_p–MHb plots demonstrated a counterclockwise hysteresis loop in all animals, indicating that a PK-PD model incorporating an effect compartment was appropriate. The $t_{1/2}k_{eo}$ values were different from the $t_{1/2}\ k_{21}$ (mean = 35.4; not shown), suggesting that the amount of drug in the effect compartment is not directly proportional to the amount of drug in the peripheral compartment of the PK model (16). The $t_{1/2}k_{eo}$ values were greater than $t_{1/2}\lambda_1$ and, with the exception of dog 9A04, less than $t_{1/2}\lambda_z$, resulting in the effect-site concentrations declining in parallel with the concentrations in the central compartment. However, due to the nonlinearity of the E_{max} model, this would result in the effect initially falling less than concentration, when the concentration is much greater than EC_{50}, linearly when the concentration is close to the EC_{50}, and exponentially when the concentration is much less than the EC_{50}.

Examination of the standard errors and confidence limits of the parameter estimates in the individual dogs showed the greatest uncertainty in the estimates of k_a, λ_1, V_1, and E_{max}. There was considerable variability about the model predicted peak WR 238605 plasma concentrations from one dose to the next in all the animals (Fig. 1, inset). This intra-individual variability was most likely due to variable drug absorption and may have contributed to the uncertainty in estimating V_1 and λ_1.

The uncertainty in estimating these PK parameters may explain why two distinct declining exponential phases could not be estimated well in dogs 9A01 and 9B07. A slow absorption rate, relative to the other rate processes, may potentially "contaminate" the underlying distribution phase, giving the appearance of a 1-COM model (17). However, when dogs 9A01 and 9B07 were fit to a 2-COM, the absorption rate constants were still faster than all other rate constants.

The uncertainty in estimating E_{max} may be due to lack of information about this parameter in the data. The peak MHb levels obtained in this study were only 7.6 to 21.3%. However, this uncertainty may not detract from the utility of the model, since the clinically relevant MHb levels are usually at the lower end of the concentration–response curve (MHb levels of 10–15%).

Although the differences in the predicted and observed peak MHb levels in two of the validation animals were less than 2% MHb, this difference in the third animal was 7.8% (Fig. 3). Dog C received a total dose similar to dog B (25 and 27 mg/kg respectively); however, less of the total dose was given as a loading dose (10 vs 18 mg/kg, respectively). The higher than predicted MHb levels in dog C could be due to greater bioavailability of the smaller loading dose. However, one would also have expected the plasma drug concentra-

Table III. Pharmacodynamic Analysis

Dog ID	$T_{1/2}$ k_{eo} (hr)	E_0 (%MHb)	E_{max} (%MHb)	EC_{50} (ng/ml)	n
9A01	116.3	0.24	31.3	398	1.71
9A03	142.9	0.10	55.3	675	1.67
9A04	134.1	0.52	34.5	724	1.57
9A05	134.4	0.56	14.6	492	2.73
9A06	134.1	0.44	39.3	694	1.68
9B07	92.5	0.61	13.1	595	2.30
Mean	123.0[a]	0.41	31.3	596	1.94
SD	22.4	0.20	15.9	128	0.47
%CV	18	49	51	22	24
SEE range	6.1–9.5	0.03–0.20	1.3–19.5	38.5–363.7	0.08–0.45

[a] Harmonic mean and jackknife standard deviation.

tions to be greater than predicted in dog C in this case. This underprediction may simply be a result of interindividual variation in the pharmacodynamics. The difference may also be due to the formation of a greater fraction of active metabolite and a lower fraction of inactive metabolite in dog C.

It is important to note the marked difference in elimination half-life between this drug and primaquine. Primaquine has an elimination $t_{1/2}$ of 4 hr (2), compared to 170 hr for WR 238605. Primaquine is typically administered at a dose of 15 mg/day for 14 days or, alternatively, 45 mg each week for 8 weeks (2). WR 238605's longer half-life may permit less frequent dosing or dosing of shorter duration when used as a radical curative antimalarial.

Cyanide is a potentially lethal poison that can debilitate within seconds and kill within minutes. In certain environments, such as a chemical warfare attack, the potential for lethal cyanide exposure is high. It is doubtful whether conventional antidotes, requiring time-consuming intravenous administration, would be effective. Prophylactic therapy may be the only practical solution (5). An effective prophylactic drug should be a long-acting, oral preparation that could be self-administered. WR 238605 may be of benefit in this situation, where a constant MHb level with infrequent dosing is desirable.

The mechanism of action of MHb formation by WR 238605 is unknown. The long delay between plasma drug concentrations and effect (mean $t_{1/2}k_{eo}$ = 123 hr) can be due to several factors. One explanation may be that one or more metabolites of WR 238605 are actually responsible for the oxidation of Hb, and there is a delay in the formation of these metabolites. Another possible explanation for the long $t_{1/2}k_{eo}$ is that there exists an equilibrium delay in the oxidizing substance (parent drug or metabolite) entering the red cell and reaching the heme group—the site of action. It is also possible that the active substance does not oxidize Hb per se, but rather, inhibits the action of MHb reductase. This would then permit endogenous oxidants to slowly form MHb with diminished compensatory reduction back to Hb.

The current model may be improved by undertaking a population PK analysis in a larger sample. This may provide better estimates of intra- and interindividual kinetic variability (18). Current efforts include identification and synthesis of potential metabolites of WR 238605. Work is also being

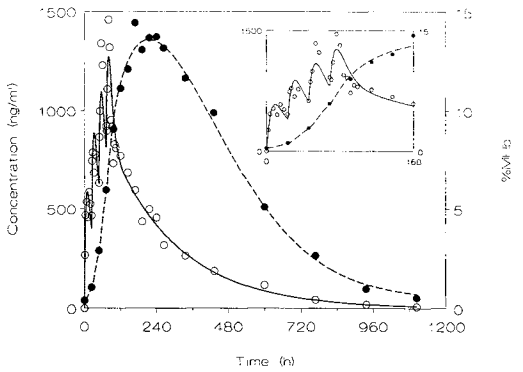

Fig. 1. Representative fits of a 2-COM PK model (——) to concentration data (○) and a sigmoid E_{max} PD model (– – –) to methemoglobin data (●) in dog 9A06.

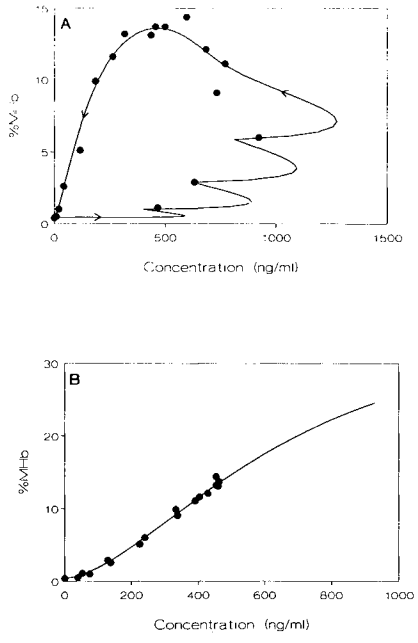

Fig. 2. Large, counterclockwise hysteresis loop in dog 9A06, obtained by plotting %MHb levels against corresponding plasma concentrations (observed data, ●; PK-PD model fit, ——) (A). Collapsed loop shows the sigmoid E_{max} concentration–effect relationship (B).

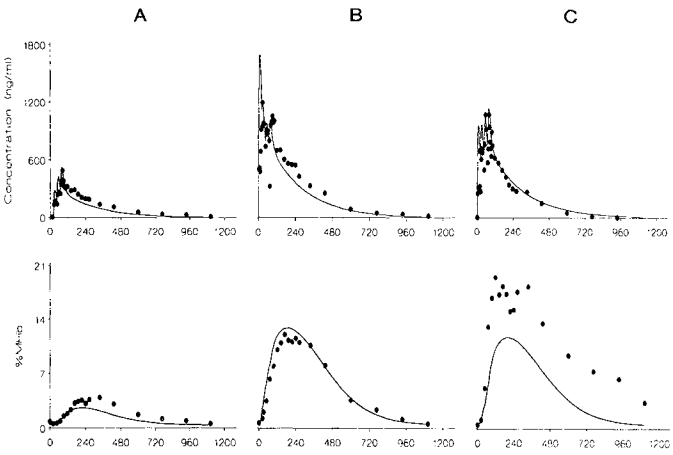

Fig. 3. Combined PK-PD model validation in three additional dogs (observed data, ●; model prediction, ——). The dogs were given three different dosing regimens totaling 9 (A), 27 (B), and 25 (C) mg/kg WR 238605.

done to determine if there is an effect of WR 238605 or one of its metabolites on MHb reductase activity.

In summary, a combined pharmacokinetic-pharmacodynamic model was developed that described the plasma concentration profile of a new candidate antimalarial and the resulting MHb levels. Further, it predicted the plasma concentrations and MHb levels and the times of their occurrence in an additional group of test animals well. This model may be useful in guiding the design of subsequent animal studies and initial phase I human studies.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Emil Lin, UCSF, San Francisco, CA, for performing the HPLC assay, Ernest Stonecipher and Rosendo Sanchez for their technical assistance, and the division of Veterinary Medicine, WRAIR.

REFERENCES

1. L. J. Bruce-Chwatt. Malaria and its control: Present situation and future prospects. *Annu. Rev. Public Health* 8:75–110 (1987).
2. D. M. Panisko and J. S. Keystone. Treatment of malaria—1990. *Drugs* 39:160–189 (1990).
3. W. Peters. *Chemotherapy and Drug Resistance in Malaria,* Academic Press, London, 1987.
4. L. T. Webster. Drugs used in the chemotherapy of protozoal infections: Malaria. In A. G. Gilman, T. W. Rall, A. S. Nies, and P. Taylor (eds.), *Goodman and Gilman's The Pharmacologic Basic of Therapeutics,* Pergamon Press, New York, 1990, pp. 988–991.
5. J. E. Bright. A prophylaxis for cyanide poisoning. In B. Ballantyne and T. C. Marrs (eds.), *Clinical and Experimental Toxicology of Cyanides,* IOP, Bristol, 1987, pp. 359–382.
6. N. S. Agar and J. D. Harley. Erythrocyte methemoglobin reductases of various mammalian species. *Experientia* 28:1248 (1972).
7. E. J. Calabrese. *Principles of Animal Extrapolation,* John Wiley & Sons, New York, 1983.
8. C. Achten, A. Benitez, and P. Lim. Assay of N4-(2,6-dimethoxy-4-methyl-5-(3-(trifluoromethyl)phenoxy)-8-quinolinyl)-1,4-pentanediamine, succinate, WR-238605AC, BK73252. Report No. 469, USAMRDC Contract No. DAMD 17-79-C-9154, SRI International, Menlo Park, CA, 1984.
9. E. T. Lin, L. Z. Benet, R. A. Upton, and W. L. Gee. Quantitation of WR 238605 in plasma and blood by high performance liquid chromatography and fluorescence detection. Study Report Number 13, USAMRDC Contract No. DAMD 17-85-C-6150, School of Pharmacy, UCSF, San Francisco, CA, March 29, 1989.
10. K. M. Dubowski. Measurements of Hemoglobin derivatives. In F. W. Sunderman and F. W. Sunderman, Jr. (eds.), *Hemoglobin, Its Precursors and Metabolites,* J. B. Lippincott, Philadelphia and Montreal, 1964, pp. 49–60.
11. J. C. Anders and H. Chung. Deficiencies and improvement of methemoglobin assay. *J. Anal. Tox.* 8:260–262 (1984).
12. D. Verotta and L. B. Sheiner. Simultaneous modeling of pharmacokinetics and pharmacodynamics: An improved algorithm. *CABIOS* 3:345–349 (1987).
13. N. H. G. Holford and L. B. Sheiner. Kinetics of pharmacologic response. *Pharmacol. Ther.* 16:143–166 (1982).
14. F. C. Lam, C. T. Hung, and D. G. Perrier. Estimation of Variance for harmonic mean half-lives. *J. Pharm. Sci.* 74:229–231 (1985).
15. B. S. Levine. Four week oral toxicity study of WR 238605 in dogs. Study Report Number 47, USAMRDC Contract No. DAMD 17-87-C-7225, University of Chicago at Illinois, July 12, 1990.
16. L. B. Sheiner, D. R. Stanski, S. Vozeh, R. D. Miller, and J. Ham. Simultaneous modeling of pharmacokinetics and pharmacodynamics: Application to *d*-tubocurarine. *Clin. Pharmacol. Ther.* 25:358–371 (1979).
17. M. Gibaldi and D. Perrier. *Pharmacokinetics,* Marcel Dekker, New York, 1982.
18. L. B. Sheiner and S. L. Beal. Evaluation of methods for estimating population pharmacokinetic parameters. I. Michaelis-Menton model: Routine clinical pharmacokinetic data. *J. Pharmacokin. Biopharm.* 8:553–571 (1980).