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**Pharmacokinetics and Pharmacokinetic-dynamic modeling of an 8-aminoquinoline
candidate anticyanide drug (WR242511)**

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Introduction: Cyanide is one of the most rapidly acting toxic compounds. With a sufficiently high dose one may die within minutes of exposure. Treatment must be rapid to be effective. Cyanide is used extensively in industry and agriculture in a variety of forms which may lead to inadvertent human exposure. Agents useful in treating cyanide intoxication include sodium nitrite, 4-dimethylaminophenol, cobalt EDTA, and hydroxycobalamin¹. Sodium nitrite and 4-dimethylaminophenol work by converting hemoglobin to methemoglobin for which cyanide has a very high affinity thus acting as a cyanide "sink". Cobalt EDTA and hydroxycobalamin act directly as cyanide chelators. Sodium thiosulfate is administered in conjunction with sodium nitrite to accelerate conversion of cyanide to thiocyanate which is nontoxic and excreted in the urine. All of the above treatments require intravenous delivery and careful monitoring by trained medical personnel.

Hydrogen cyanide is considered a serious chemical warfare threat because it can be delivered to the battlefield in concentrations sufficiently to cause extensive morbidity and mortality². In military situations the administration of any of the known antidotes would be virtually impossible because of the number of casualties, the short time span in which the antidote needs to be delivered, and the limitations of MOPP. A prophylactic drug for cyanide poisoning would be the treatment of choice to avert mass casualties. The ideal drug would be effective in the majority of the population being treated, the dosing rate would be daily or less frequent, it would have minimal side effects and would not interfere with aerobic and anaerobic work necessitated in the course of military duties. In addition it would be devoid of carcinogenic and mutagenic potential.

WR242511, a 8-aminoquinoline primaquine analog, has been shown to be a significant methemoglobin former in previous studies. In this study both the pharmacokinetics and pharmacodynamics of WR242511 in dogs are studied and two different pharmacokinetic - pharmacodynamic models are described. The single dose models are then used to predict steady state methemoglobin levels in multidose studies.

94-08721



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Materials and Methods:

Drug: WR242511 (8-[(4-amino-1-methylbutyl)amino]-2-methoxy-5-hexoxy quinoline succinate was synthesized by Ash-Stevens Inc. Bottle number 05816 was used in this study. WR256408 was utilized as internal standard for HPLC analysis and was also synthesized by Ash-Stevens Inc. All other materials used were of HPLC grade and were obtained commercially. Gelatin capsules for the oral formulations were size 000 obtained from Parke-Davis. The intravenous formulation was made in 100% polyethylene glycol (average molecular weight 200) obtained from Sigma, St. Louis Mo. and was filter sterilized utilizing Pro-X™ filters (.45 um Hydrophilic cellulose acetate membrane) obtained from Lida manufacturing corp., Kenosha WI. Both WR242511 and WR256408 and their methanolic solutions used in the HPLC analysis were kept in amber bottles at 10 degrees Celsius.

Animals: 10 healthy male beagles weighing between 8 and 12 Kg were obtained from Hazelton Research Laboratories, Inc. (Cumberland, Va.). They were cared for by our veterinary staff and were certified healthy and had normal laboratory baseline tests. 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 NIH 85-23. They were housed in runs measuring 4 * 10 feet. The environment was controlled within average of 68 - 72 degrees Fahrenheit and 40 - 60 % humidity. They were provided a measured amount of purina dog chow daily and water ad-libitum.

Dosing: Each dog received doses of 3.5 mg/kg iv and 7 mg/kg po and iv in the single dose studies and a loading dose between 2 - 8 mg/kg po and maintenance doses between .5 - 2 mg/kg po every 48 hours in the multiple dose studies as the succinate base. The animals daily weight was used for dosing and all animal were doses between 0845 and 0900 hours. The oral and iv doses were made daily prior to dosing.

Sampling: Blood samples were obtained for determination of plasma drug concentrations and methemoglobin levels over 7 - 10 days. Blood samples for the oral dosing were obtained at 0, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 30, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hours. Blood samples for the intravenous dosing were obtained at 0, 1, 3, 5, 10, 15, 20, and 30 minutes and at the same hour time points as the oral dosing. Samples were collected from the cephalic or saphenous vein in heparinized 3 ml syringes and placed on ice. 100 mcl of whole blood was used for methemoglobin measurements and were made within 30 minutes of sampling. The remainder of the blood sample

was centrifuged in a Eppendorf 5413 centrifuge for 6 minutes. The plasma was taken off and stored at -20 degrees Celsius until used for the determination of drug concentration.

Analytical: Plasma WR242511 levels were determined by HPLC as described by Marino et al³. Briefly the method used a waters intelligent sample processor 712, waters model 6000 HPLC pump, a 5 micron cyano precolumn and a Waters uBondapak™ 86688 cyano column (10µM, 3.9mm * 150mm). The detector was a BAS electrochemical detector using a single glassy carbon electrode set in the oxidative mode at .5 volts. The recirculating mobile phase coincided of a 70:30 mixture of acetonitrile and sodium acetate 50 mM pH6.0 with 1 mM EDTA with a flow rate of 2 ml/minute. 250 µl plasma samples were extracted with 250 µl of a 3:1 (V:V) mixture of ter-butyl-methyl-ether and 2-propanol after the addition of 312.5 ng of WR256408 as the internal standard. 25 - 50 µl of the organic layer was injected on the column. The limits of quantitation of WR242511 was 25 ng/ml as the succinate salt. Each animals samples were processed separately with a standard curve and pre and mid run validation samples at 50 and 1000 ng/ml (n=4).

Methemoglobin was determined on each sample using the OSM3 Hemoximeter® automated analyzer, Radiometer (Copenhagen), with settings for dog hemoglobin. The method is based on spectrophotometric changes in hemoglobin and 10 µl samples of heparinized blood were injected per sample. The analyzer has a limit of detection of .5% methemoglobin and a C.V. of .4% throughout its range of detection.

Data Analysis: Compartmental and noncompartmental analysis was done on the pharmacokinetic data. The area under the curve was calculated from the nonlinear fitted curve from RSTRIP extrapolated to infinity. The area under the moment curve was calculated in the same way. Both oral and intravenous clearances were calculated as follows

$$CL_{\text{oral, intravenous}} = \text{Dose} / \text{AUC}$$

Volume of distribution at steady state, Kabsorption, and bioavailability F were calculated as follows.

$$V_{ss} = CL * MRT$$

$$F = \text{AUC}_{\text{oral}} * \text{Dose}_{\text{intravenous}} / \text{AUC}_{\text{intravenous}} * \text{Dose}_{\text{oral}}$$

Compartmental analysis was also done on the kinetic data utilizing a one or two compartment model for each experiment with weighted least squares analysis using RSTRIP (Micromath Scientific software, Salt Lake City, Utah). The model that fit the data best as determined by the model selection criteria, visual fit of the data, 95% confidence intervals for each estimated parameter, and residual analysis was selected for each animal. The following parameters were estimated using the compartmental approach $K_{\text{absorption}}$, $K_{\text{elimination}}$, $K_{\text{distribution}}$.

A nonparametric PD analysis of the concentration-Methemoglobin data was performed to guide selection of the appropriate model and the initial choice of K_{eo} . K_{eo} describes the rate of loss from the effect compartment and determines the temporal delay between plasma drug concentration and effect. This analysis was performed on MATHCAD (MathSoft, Inc. Cambridge, Mass.) and is shown in figure 1 for a sample dog.

A 1 and 2 compartment open model with elimination from the central compartment and first order absorption with the effect compartment linked to the central compartment was written using MKMODEL (Biosoft, Milltown, N.J.). For the plasma concentration for the one and two compartment models (oral and intravenous respectively) was parameterized as follows

One compartment / Oral $\text{Conc}_t = F * K_a * \text{Dose} * (e^{(-K_a * t)} - e^{(-K_e * t)}) / ((K_e - K_a) * V_d)$

Two compartment / IV $\text{Conc}_t = \text{Dose} * (K_{21} - K_{12}) * e^{(-K_1 * t)} / (V_p * (K_{12} - K_{10})) +$
 $\text{Dose} * (K_{21} - K_{12}) * e^{(-K_{10} * t)} / (V_p * (K_{12} - K_{10}))$

The effect site concentration C_e was used in a sigmoid Emax model to describe the effect (% Methemoglobin).

One Compartment / Oral $C_e = F * K_a * \text{Dose} * e^{(-K_a * t)} / ((K_e - K_a) * (K_{\text{eo}} - K_a)) +$
 $F * K_a * \text{Dose} * e^{(-K_e * t)} / ((K_a - K_e) * (K_{\text{eo}} - K_e)) +$
 $F * K_a * \text{Dose} * e^{(-K_{\text{eo}} * t)} / ((K_a - K_{\text{eo}}) * (K_e - K_{\text{eo}}))$

Two Compartment / IV $C_e = (K_{21} - K_{12}) * \text{Dose} * e^{(-K_1 * t)} / ((K_{12} - K_{10}) * (K_{\text{eo}} - K_{12})) +$
 $(K_{21} - K_{12}) * \text{Dose} * e^{(-K_{10} * t)} / ((K_{12} - K_{10}) * (K_{\text{eo}} - K_{10})) +$
 $(K_{21} - K_{10}) * \text{Dose} * e^{(-K_{\text{eo}} * t)} / ((K_{12} - K_{10}) * (K_{10} - K_{\text{eo}}))$

Effect equation for both
one and two compartment
models

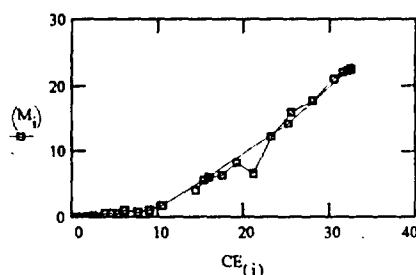
$$E = E_{\text{max}} * (C_e)^{\text{Hill}} / ((EC50)^{\text{Hill}} + (C_e)^{\text{Hill}})$$

$K_e = .045$ $K_a = .73$ $K_{eo} = .004$ $E_{max} = 100$ $EC50 = 43$
 $Dose = 7000$ $V_d = 15$ $n = 2.5$

$$CE_{(i)} = \frac{(K_e - K_{eo}) \cdot e^{-(K_a \cdot t_i)} + (K_{eo} - K_a) \cdot e^{-(K_e \cdot t_i)} + (K_a - K_e) \cdot e^{-(K_{eo} \cdot t_i)}}{(K_e - K_{eo}) \cdot (K_{eo} - K_a) \cdot (K_a - K_e)} \cdot \frac{1 \cdot K_a \cdot K_{eo} \cdot Dose}{V_d}$$

$K_{eo} = .004$

$$MP_i = \frac{E_{max} \cdot (CE_i)^n}{EC50^n + (CE_i)^n}$$



$K_e = .045$ $K_a = .73$ $K_{eo} = .002$ $E_{max} = 100$ $EC50 = 43$
 $Dose = 7000$ $V_d = 15$ $n = 2.5$

$$CE_{(i)} = \frac{(K_e - K_{eo}) \cdot e^{-(K_a \cdot t_i)} + (K_{eo} - K_a) \cdot e^{-(K_e \cdot t_i)} + (K_a - K_e) \cdot e^{-(K_{eo} \cdot t_i)}}{(K_e - K_{eo}) \cdot (K_{eo} - K_a) \cdot (K_a - K_e)} \cdot \frac{1 \cdot K_a \cdot K_{eo} \cdot Dose}{V_d}$$

$K_{eo} = .002$

$$MP_i = \frac{E_{max} \cdot (CE_i)^n}{EC50^n + (CE_i)^n}$$

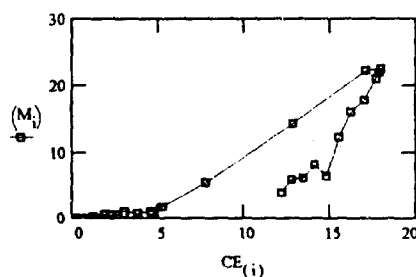


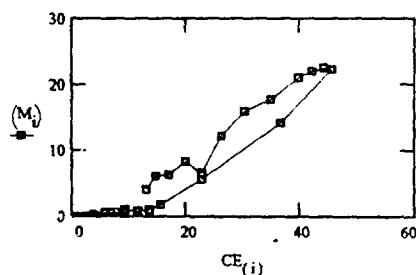
Figure 1

$K_e = .045$ $K_a = .73$ $K_{eo} = .006$ $E_{max} = 100$ $EC50 = 43$
 $Dose = 7000$ $V_d = 15$ $n = 2.5$

$$CE_{(i)} = \frac{(K_e - K_{eo}) \cdot e^{-(K_a \cdot t_i)} + (K_{eo} - K_a) \cdot e^{-(K_e \cdot t_i)} + (K_a - K_e) \cdot e^{-(K_{eo} \cdot t_i)}}{(K_e - K_{eo}) \cdot (K_{eo} - K_a) \cdot (K_a - K_e)} \cdot \frac{1 \cdot K_a \cdot K_{eo} \cdot Dose}{V_d}$$

$K_{eo} = .006$

$$MP_i = \frac{E_{max} \cdot (CE_i)^n}{EC50^n + (CE_i)^n}$$



Emax was set at 100% for the following reasons. The effect of this drug and similar compounds has been shown in toxicologic studies to produce near 100% methemoglobin levels. In-Vitro studies with other classes of compounds have shown similar high methemoglobin levels. Since the effect of the drug is to promote the oxidation of hemoglobin to methemoglobin and there is no evidence for hemoglobinopathies with different susceptibilities for methemoglobin production in these animals any difference in methemoglobin production would most probably be due to each individual's elimination and metabolism of the compound. The simultaneous fitting of the PK-PD data was based on the standard goodness of fit criteria to include Visual fit of the curves, residual analysis and the log likelihood and the Schwartz criteria.

Simulations of a PK-PD model for the data were also done using STELLA (High Performance Systems, Inc. Lyme N.H.). The model utilized the first order absorption coefficients and the first order elimination coefficients obtained from compartmental analysis for the PK part of the model. The simulation assumed a first order conversion to active metabolite which was a small fraction of the elimination constant. This metabolite then had its own elimination constant and a first order entry into the red blood cell. With the red blood cell being the effect site an Emax model described the effect (% methemoglobin). The methemoglobin was then reduced back to hemoglobin via Michaelis-menton kinetics as modeled from the red blood cell enzyme methemoglobin reductase. The simulation was fit as follows. The concentration time data were fit to confirm the appropriateness of the PK parameters from compartmental analysis. Next the two parameters Kelimination and Kmetabolism were altered to fit the methemoglobin data by sensitivity analysis (i.e sweeping over a range of values) but keeping the sum the same as the overall elimination parameter Kelimination obtained from the compartmental analysis. The model is shown in figure 3.

Model Validation: Four animals were used to confirm the appropriateness of the PK-PD model and the PK-PD simulation. Each dog was given a multi-dose regimen to produce a steady methemoglobin level as determined by the simulation model. Each dog received individualized doses to produce different steady state levels of methemoglobin but each had a loading dose and a maintenance dose at 48 hour intervals. Predicted drug concentrations and predicted methemoglobin % were compared to measured drug levels and measured methemoglobin. The dosing regimens were all different than the regimens to develop the model.

Results: All animals tolerated the doses well except dog 4632 who had some vomiting 2 hours after oral dosing with 7 mg/kg. All animals had hemolysis after receiving both oral and intravenous dosing which cleared within 48 hours of dosing and did not produce any gross hemoglobinuria. All dogs who developed methemoglobin levels above 10% demonstrated a cyanotic appearance of their tongues, gums, and sclera.

The WR242511 concentration-time data with intravenous formulations best fit a 2-compartment model and all experiments with oral formulations best fit a 1 compartment model. These values are found in table 1. Also in table 1 are the pharmacokinetic parameters found from the non-parametric analysis. WR242511 was found to have a elimination T_{1/2} of 32 +/- 10 hours, a distribution T_{1/2} of 9 +/- 2 min, a V_{ss} of 14.6 +/- 2.2 L / Kg, a clearance of .37 +/- .17 L / Kg * hour and an oral clearance of .65 +/- .28 L / Kg * hour. Peak WR242511 plasma concentrations were found within 12 hours for the oral dosing and peak methemoglobin was found to be markedly delayed from the peak serum drug concentration at 12 hours at the earliest. The delay between methemoglobin production and drug plasma concentration produced a counterclockwise hysteresis loop as demonstrated for all the animals with both oral and intravenous dosing (figure 2). Most dogs except dogs 4606 and EBWAG appeared to have higher methemoglobin responses with the oral formulation as measured by peak methemoglobin and the methemoglobin AUC.

The nonparametric model suggested a sigmoid E_{max} model for the methemoglobin response (figure 1). In none of the animals did the collapsed hysteresis loops suggest an E_{max} so E_{max} was chosen as 100% for the reasons described in data analysis. All parameters from the PK-PD modeling with MKMODEL are shown in table 2. The T_{1/2} Keo was 144 +/- 43 hours for both oral and intravenous dosing and the hill coefficient was 2 +/- .4 for both oral and intravenous dosing but was consistent in each animal for both dosing routes. The EC₅₀ was the only value to show changes in several animals. EC₅₀ was found to be relatively high in the animal who was a hyporesponder with respect to methemoglobin production and relatively low in the animal who was sensitive to the drug. In all the animals except the two EBWAG and 4606 the EC₅₀ for the oral was lower than for the intravenous doses.

The STELLA simulation utilized the pharmacokinetic data from the compartmental analysis and fit the methemoglobin data to the model parameters. All that was altered to fit the simulation were the parameters Ke and Kmetabolism for WR242511.

Both the PK-PD model and STELLA were used to predict steady state methemoglobin levels with the multiple dose experiments. The differences for each model averaged with 5 % and are shown in figure 4 for one animal.

Experiment PD601 7 mg / kg PO

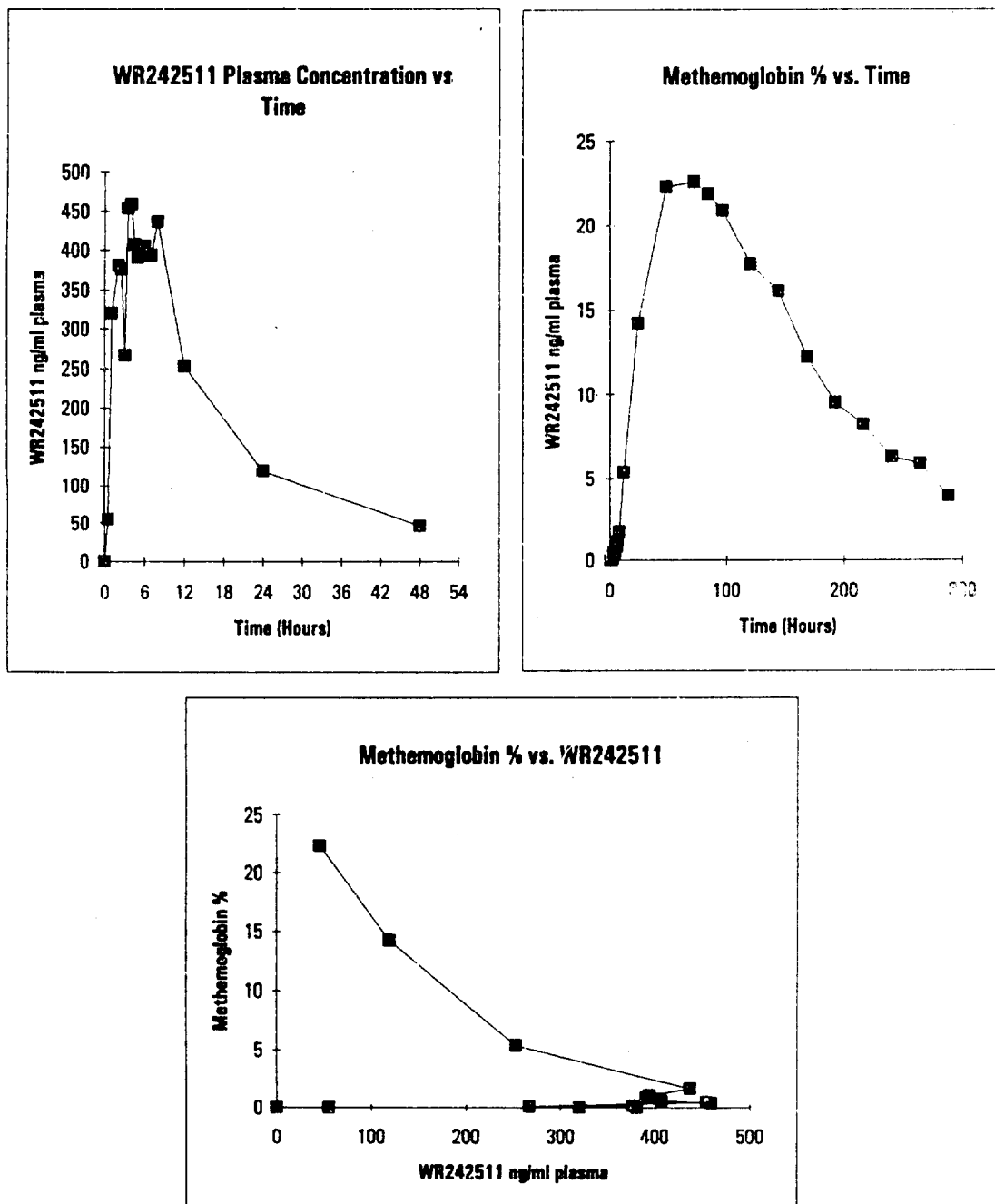


Figure 2

Stella Model of PK - PD WR242511

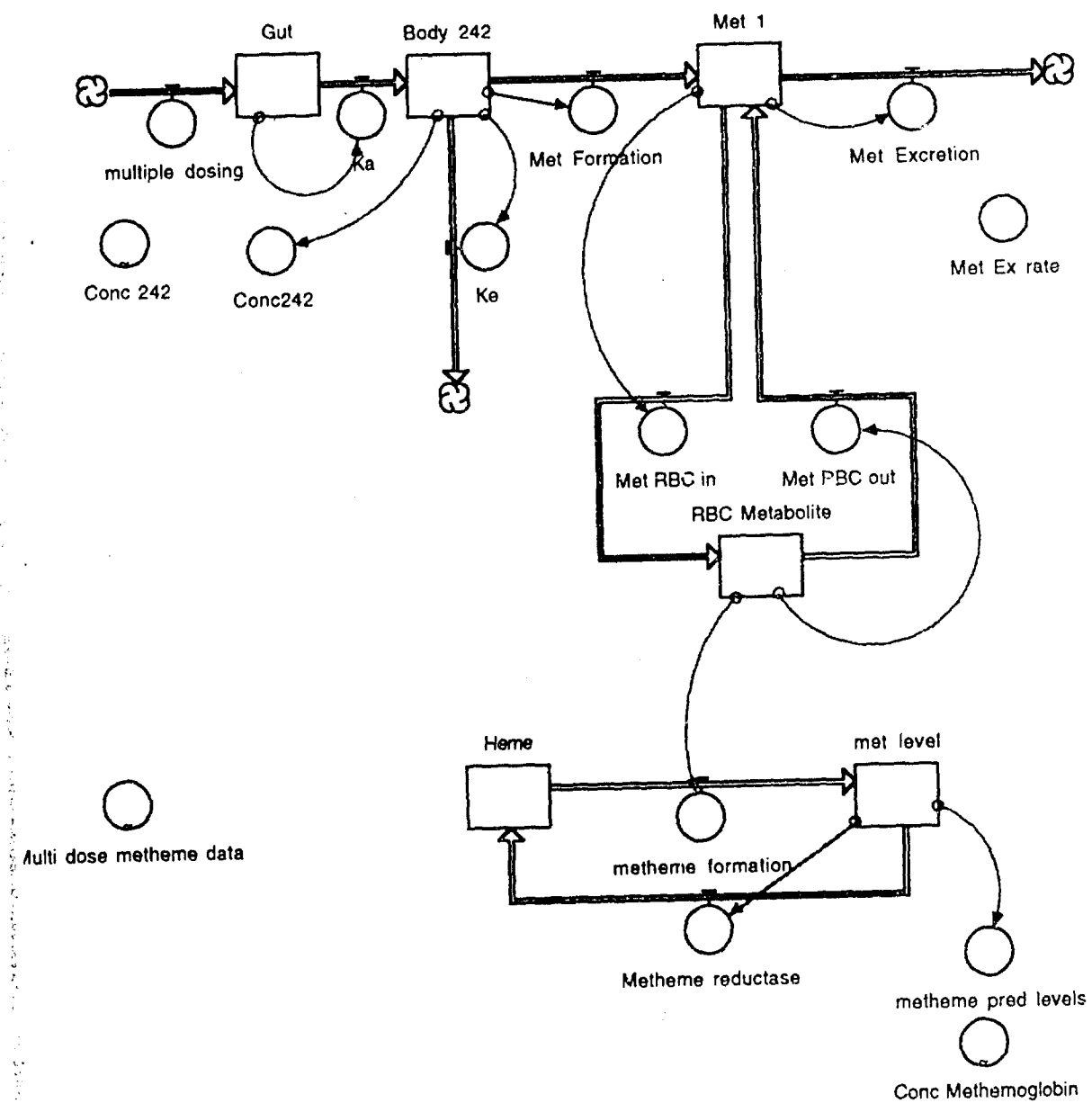


Figure 3

Model Predictions for multidosing with WR242511 in dog EEWAA
Measured Methemoglobin, MKModel and Stella predictions

Methemoglobin %

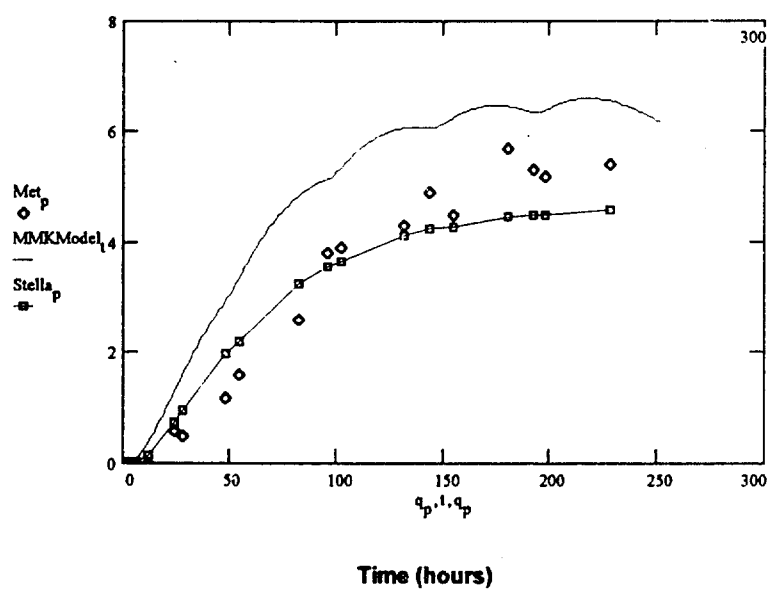


Figure 4

Table 1

			Summary of Experiments at different Dosing Levels										WR242511					
Animal	Experiment #	Dose	Peak Levels	Time to	WR242 T 1/2	% Methemoglobin	Time to peak	AUC WR242	MRT	V:z	T 1/2	CL (L / Kg *hr)	T1/2 Distribution					
F4842	P0610	3.5MG/KG IV	381ng/ml	N.A.	48 hrs	2.6%	120 hrs	13315	67	17.61	46.43	0.26	0.19					
	P0612	3.5MG/KG IV	443ng/ml	N.A.	30 hrs	10.1%	72hrs	9838	43	15.30	29.80	0.36	0.15					
	P0613	3.5MG/KG IV	384ng/ml	N.A.	38 hrs	8.3%	72 hrs	11746	54	16.09	37.42	0.30	0.19					
F4806	P0625	7MG/KG IV	1126ng/ml	N.A.	14 hrs	15.5%	72 hrs	9455	20	14.81	14.06	0.74	0.07					
	P0616	7MG/KG IV	1244ng/ml	N.A.	34hrs	14.4%	96hrs	25733	49	13.33	33.96	0.27	0.12					
	P0617	7MG/KG IV	988ng/ml	N.A.	35hrs	7.2%	120hrs	22609	51	15.79	35.34	0.31	0.15					
	P0618	7MG/KG IV	1034ng/ml	N.A.	17 hrs	18.8%	72 hrs	12028	24	13.97	16.63	0.58	0.10					
	P0619	7MG/KG IV	1283ng/ml	N.A.	24 hrs	16.3%	96 hrs	23164	34	10.27	23.56	0.30	0.15					
FEEWAA	P0624	7MG/KG IV	1544ng/ml	N.A.	53 hrs	13.2%	119 hrs	32046	76	16.60	52.72	0.22	0.20					
Apparent Oral Clearance																		
F4806	P0615	7MG/KG PO	347ng/ml	4 hrs	21 hrs	13.8%	72 hrs	12798	32			0.55						
	P0680	7MG/KG PO	430 ng/ml	4 hrs	21.3 hrs	15.2%	84 hrs	14578	33			0.48						
	P059	7MG/KG PO	172 ng/ml	12 hrs	51 hrs	5.0%	168 hrs	11645	77			0.60						
FEBWAG	P0601	7MG/KG PO	486 ng/ml	3.5 hrs	11.3 hrs	22.6%	48 hrs	9420	19			0.74						
	P0602	7MG/KG PO	244ng/ml	8 hrs	13.6 hrs	15.0%	72-84 hrs	6065	23			1.15						
	P0614	7MG/KG PO	337ng/ml	12 hrs	38 hrs	11.5%	84 hrs	20025	56			0.35						
Bioavailability (SD) %			68% (37)															
Clearance iv (SD)			L / (kg *hr)															
Clearance po (SD)			L / (kg *hr)															
Vss (SD)			L / Kg															
T 1/2 (SD)			hrs															
T 1/2 Data			150(0.04)															
			9(2)															

Pharmacokinetic - Pharmacodynamic modeling of WR242511 in Dogs

Experiment #	Dog	Dose	Route	K _{abs} (hrs ⁻¹)	K ₁₂ (hrs ⁻¹)	K ₂₁ (hrs ⁻¹)	K _{el} (hrs ⁻¹)	K _{el} (hrs ⁻¹)	EC ₅₀ (ng/ml)	HL
P0617	4642	7 mg/kg	IV		4.28	0.013	0.008	1.84	100	576
P0618	4642	7 mg/kg	PO	0.4		0.011	0.007		100	415
P0619	4642	3.5 mg/kg	IV		3.03	0.015	0.004	1.06	100	514
P0616	4632	7 mg/kg	IV		8.01	0.02	0.006	2.43	100	184
P0600	4632	7 mg/kg	PO	0.5		0.032	0.004		100	83
P0619	ECWAG	7 mg/kg	IV		3.08	0.022	0.006	1.94	100	262
P0602	ECWAG	7 mg/kg	PO	0.5		0.034	0.004		100	44
P0613	ECWAG	3.5 mg/kg	IV		3.71	0.019	0.008	2	100	136
P0618	EWAG	7 mg/kg	IV		7.49	0.041	0.004	2.83	100	86
P0601	EWAG	7 mg/kg	PO	0.75		0.043	0.004		100	87
P0612	EWAG	3.5 mg/kg	IV		0.24	0.039	0.008	2.25	100	84
P0614	EEWAA	7 mg/kg	PO	0.3		0.019	0.005		100	231
P0624	EEWAA	7 mg/kg	IV		10.07	0.015	0.004	2.33	100	290
P0615	4606	7 mg/kg	PO	0.39		0.039	0.006		100	101
P0625	4606	7 mg/kg	IV		7.97	0.033	0.003	2.35	100	54
			Mean	0.47	0.06	0.03	0.006	2.18	100.00	205.73
			S.D.	0.16	2.41	0.01	0.001	0.36	0.00	174.26

Table 2

11

Discussion: This is the first combined PK-PD model and simulation of the candidate anticyanide compound WR242511. The model describes the plasma concentrations, and methemoglobin formed in each individual animal. The models were subsequently validated in animals with multiple dose to produce a steady state methemoglobin level. The models may provide insight into possible hypothesis for interanimal variability and intraanimal variability for different dosing routes.

The Cp-MHb plots demonstrate counterclockwise hysteresis for all animals and all dosing routes suggesting that an effect compartment is appropriate. The $T_{1/2}$ Keo was different from $T_{1/2}$ K21 suggesting that the amount of drug in the effect compartment is not directly proportional to the amount in the peripheral compartment. $T_{1/2}$ Keo were greater than $T_{1/2}$ Ke resulting in effect site concentrations falling much slower than concentrations in the central compartment.

The discrepancy in the EC50 values between oral and intravenous dosing within the same animal suggests that there is first pass metabolism which converts WR242511 in an active metabolite. If an increased level of active metabolite were formed with the oral route than for each plasma level of parent compound there would be an increased amount of active metabolite. The amount of active substance would therefore be higher for oral dosing for any equivalent plasma level of parent as compared to intravenous dosing. This is also corroborated by examining the ratios of AUC MHb to AUC WR242511 for any individual animal and route. For all the animals except 4606 the ratios are higher for oral than for IV dosing suggesting that an active first pass metabolite is formed with oral dosing. In animals 4606 and EBWAG the EC50 are not significantly different by route but the animal showed a similar response to both IV and oral doses. This type of analysis was used to explain the differences in Verapamil PK-PD with oral and IV doses⁴ as there is selective metabolism of the active enantiomer to an inactive metabolite with oral dosing. This type of approach has also been used with other drugs. With WR242511 one hypothesis is that there is first pass metabolism to an active compound.

This analysis is supportive of the hypothesis that a metabolite is responsible for methemoglobin formation. In a previous study of a related 8-aminoquinoline compound WR238605 utilizing simultaneous PK-PD analysis demonstrated a clockwise hysteresis with a long delay between plasma drug concentrations and effect ($T_{1/2} K_{eo}$ 123 hours). This study was comprised of only oral dosing to animals. The model was unable to discriminate between the action of drug being delayed due to metabolite formation or to an equilibrium delay at the level of formation of methemoglobin. Another possibility exists in that the active moiety may act to inhibit methemoglobin reductase as thus show a delay in production of methemoglobin as it naturally accumulates and decrease the clearance of methemoglobin by methemoglobin reductase inhibition prolonging the duration of effect. Further work on the in-vitro effects of WR242511 on methemoglobin reductase will help answer this question.

In summary a combined pharmacokinetic-pharmacodynamic model and simulation were developed to describe the plasma concentrations of WR242511 and the resulting MHb levels. Further the model showed a discrepancy in EC50 for oral and intravenous dosing within animal supporting the hypothesis that an active metabolite is formed from first pass metabolism. The simulation was able to model the drug concentration and MHb data with the production of an active metabolite. This simulation was applicable to all the animals with only altering the rates of metabolite formation. This effect could not be achieved by simply modeling the parent compound. Both models were predictive in multidose studies designed to, produce steady state methemoglobin levels. These models may be useful in helping to optimize experiments design to identify active metabolites and their mechanism of action.

¹ Klassen, C.D. Nonmetallic environmental toxicants: Air pollutants, solvents and vapors, and pesticides in *The Pharmacological Basis of Therapeutics*, 8th edition, 1990, pg 1630.

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