The intravenous pharmacokinetics of diminazene in healthy dogs

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ABSTRACT

Diminazene remains one of South Africa's most commonly used antiprotozoal agents for the management of babesiosis in dogs . Although the drug has been on the market for over 40 years, its intravenous pharmacokinetics are poorly known. To better understand the pharmacokinetics of the drug Berenil®, it was reconstituted in sterile water and administered intravenously to 6 adult German shepherd dogs. All 6 dogs demonstrated the previously described secondary peak in the plasma concentration *versus* time profile. The plasma pharmacokinetics for diminazene are described by both non-compartmental and compartmental models. From non-compartmental analysis, the area under curve to the last sample point (AUC_{last}), clearance (CL) and volume of distribution (V_z) were 4.65 ± 1.95 ng/mℓ/h, 0.77 ± 0.18 ℓ/kg/h and 2.28 ± 0.60 ℓ/kg, respectively. For compartmental modelling, the plasma concentrations were fitted to both a 2-compartmental open model and a recirculatory enterohepatic model. From the recirculation model, the rate of release and re-entry into the central compartment varied markedly with the rate of release from the gall bladder (T_{tom}) being estimated at 27 ± 20.90 h. Once released, drug re-entry into the central compartment was variable at 9.70 ± 5.48 h. With normal biliary excretion time being about 2 h, this indicates that the redistribution cannot be occurring physiologically from the bile. Although it was not possible to identify the site from which sequestration and delayed release is occurring, it is believed that it is most likely from the liver. The study therefore showed that the secondary peak described for the pharmacokinetics of intramuscular administered diminazene in the dog is not related to biphasic absorption.

Keywords: Berenil®, dog, enterohepatic, intravenous, pharmacokinetics, PK40, recirculation.

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INTRODUCTION

Babesiosis is an economically important tick-borne disease that affects a wide range of vertebrate hosts in both the tropical and subtropical regions of the world[17]. This protozoan parasite infects most domestic animals in South Africa, ranging from dogs to cattle. It is responsible for major financial losses to cattle farmers and high cost of clinical care to owners of companion animals[4,5]. *Babesia canis* can cost South African pet owners millions of rands in veterinary costs and/or preventative measures[5].

The parasite, which develops within erythrocytes, induces moderate to severe haemolysis with subsequent anaemia[8,17]. In very severe infection this may result in death due to hypoxia, while in less severe cases the disease is usually associated with ischaemic changes[8]. Irrespective of the stage of the disease, dogs with babesiosis are treated with 1 of the 3 antibabesial drugs available in South Africa. These are diminazene, imidocarb and the dye trypan blue, of which diminazene is probably the most commonly used[3].

Diminazene has been used in South Africa since the early 1960s as Berenil® (Hoescht, now Intervet) and has dominated the market, as the other commonly used effective agent at the time, phenamidine isothionate (Phenamidine, May-Baker), also an aromatic diamidine, caused severe allergic reactions, collapse and death in the treated animals[2,15]. Although diminazene has largely become the preferred therapeutic choice in the treatment of babesiosis in dogs, it is toxic and has been associated with cerebral toxicity on occasion in both sick and healthy dogs when used at both the recommended (3.5 to 4.2 mg/kg) or supra-therapeutic doses[5,14,15].

With diminazene featuring widely in small animal therapeutics, the pharmacokinetics of this drug have been described when applied by various routes. In one study the intravenous deposition of diminazene in dogs, at a dose of 3.5 mg/kg[1], was fitted to a 2-compartmental open model. According to this study the drug was both rapidly excreted and distributed. In another study, the tissue kinetics of diminazene, in dogs, followed the recommended intramuscular route of administration at a dose of 3.5 mg/kg[16]. In contrast to the 1st study[16], no drug was detectable in the plasma as early as 48 h post-administration. The drug was, however, present in very high concentrations in the liver and kidney at 48 h with the concentration in the liver being twofold higher, and was still present in these tissues 10 days post-administration.

In a more recent study the disposition of diminazene following intramuscular administration was best described by a 2-compartmental open model[11]. One of the main findings was the rapid absorption of the drug, which, to a large extent, resembled intravenous administration. There was also a secondary peak at 100 h post-administration, similar to that described in cattle[7] and goats[11] but notably absent in dogs in previous studies[1]. It was suggested that the secondary peak could have resulted from the sequestration and recirculation of the drug[11], or possibly even from a delayed secondary absorptive phase[7]. To test these hypotheses, a comprehensive pharmacokinetic study of dogs was undertaken over a period of 144 hours after the intravenous administration of Berenil.

MATERIALS AND METHODS

Animals

Six vasectomised German shepherd dogs of similar age (1.5 to 3 yrs) and weight (30 ± 2.5 kg) were used. The dogs were obtained from the police dog school, where it is routine to vasectomise dogs to prevent unwanted breeding without the loss of aggression. The dogs were housed under tick-free conditions for a 3-month period prior to the start of the trial. Additional tick control was effected by the topical monthly application of fipronil (Frontline, Merial) according to the manufacturer's instructions, with the last dose being administered at least 4 weeks before the commencement of the study.

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Full clinical examinations, full blood counts and serum biochemistry (ALT, ALP, urea, creatinine, albumin, globulin, Na^+, K^+ and Ca^{2+}) were performed to confirm the animals' health status 1 day prior to the study. Teflon catheters (Jelco, Johnson and Johnson) were inserted in both cephalic veins prior to commencement of the study. Animals were fed a commercial dog diet and had free access to water. Food was withheld from the night before treatment until 4 hours after treatment.

Treatment

Diminazene (Berenil®, Intervet) was reconstituted in sterile water to a 4.5 % m/v concentration. A dose of 4.2 mg/kg of the reconstituted solution was administered to each dog as a once-off bolus into only 1 of the 2 catheters.

Sample collection

Blood (2.5 mℓ) was collected prior to treatment and at 0.08, 0.166, 0.25, 0.30, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72, 96, 120 and 144 h after treatment. Blood samples were not collected from the same catheter into which the drug was administered. For the first 12 hours, prior to sample collection the first 0.5 mℓ of blood was discarded. Subsequent to sample collection 0.3 mℓ of (40 IU/mℓ) of sodium heparin in saline solution was instilled into the catheter and the port sealed. During the first 12 hours blood was collected from catheters for into non-heparinised syringes (Braun) and immediately transferred to pre-marked heparinised vacutainer tubes (BD Vacutainer Systems). Subsequently all samples were collected directly into the vacutainers. Within 30 min of collection the samples were centrifuged at 3000 rpm (1200 *g*) for 15 min (Beckman Coulter,), the plasma transferred to polycarbonate tubes (NUNC, Denmark) and stored at –25 °C until analysed. All samples were analysed within 1 month of the last sample collection

Diminazene assay

Analysis was performed using a previously validated HPLC method[11]. The active ingredient was extracted from the plasma samples with C18 solid phase extraction (SPE) cartridges and eluted with 2 mℓ AcCN/OSA/AcAc. After drying under a stream of nitrogen gas at 60 °C, reconstitution was performed with 300 μℓ AcCN:H_2O (20:80). The reconstituted samples were placed in vials and 100 μℓ was injected onto a Waters Bondapack C18 reverse phase column. Imidocarb was added as internal standard. A Beckman System Gold liquid chromatograph (Beckman Coulter, Fullerton CA, USA), equipped with a model 126 dual solvent pump, a model 508 autosampler, and a model 168 programmable diode array detector was employed. Diminazene was detected at 370 nm and imidocarb at 254 nm (wavelength-switching between the 2 peaks). Samples were eluted isocratically with AcCN:0.005 M octane sulphonic acid sodium salt, containing 1 % glacial acetic acid (30:70), at a flow rate of 1 mℓ/min. Freeze–thaw stability testing was first undertaken to show that the diminazene samples were stable when stored at –25 °C.

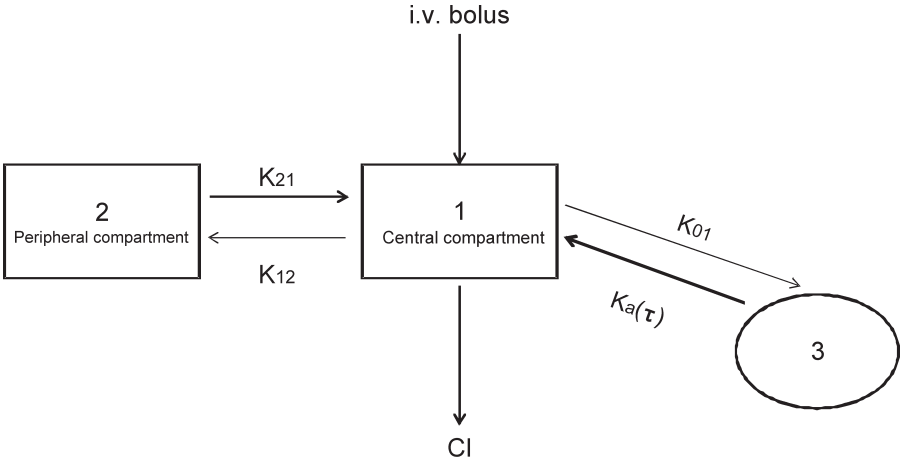

Fig 1: **Schematic representation of the enterohepatic recirculation model** (Model PK40) **to which the data were fitted. K_{21}, K_{12}, K_{01}, K_a represent the rate constants for the movement of the drug from the central compartment to the relevant peripheral compartment and back. τ represents the delay in the movement of drug from compartment 3 into the central compartment and may represent natural delay in gall bladder excretion (T_{tom}) or delayed redistribution. Cl represents whole body clearance.**

Pharmacokinetic analysis

All curve fitting was undertaken in Kinetica 4.4 (Thermo Electron Corporation). For non-compartmental modelling, the elimination rate constant (λ) and the elimination half-life ($T_{½}$) were calculated from the terminal phase. The concentration at zero hours (Cp_0) was extrapolated by linear regression. The area under the plasma concentration *vs* time curve (AUC) was obtained by the linear trapezoidal rule, until the last measurable concentration (C_{last}), with extrapolation to infinity (AUC_{inf}) using the elimination rate constant (C_{last}/λ). Total body clearance (Cl), volume of distribution (V_z), and the mean residence time (MRT) were calculated using standard equations.

For 2-compartmental modelling, parameters estimated were the macroconstants: distribution constant (α), elimination constant (β), intercept of the distribution phase (A), the intercept of the elimination phase (B) and volume of distribution (V_z). The microconstants K_{01}, K_{10}, K_{21} were derived from the macroconstants. Secondary parameters, including area under curve (AUC), area under the moment curve (AUMC), the half-life of elimination ($T_{½\beta}$), half-life of distribution ($T_{½\alpha}$), biological half-life ($T_{½lz}$) and mean residence time (MRT) were derived from the primary parameters. All plasma profiles were analysed to the last observable plasma concentration except for dog 3 whose the curve was truncated at 6 h due to poor curve fitting. The best fit equation at time (t) was defined as:

$$Cp(t) = A*e^{-\alpha*t} + B*e^{-\beta*t}, \quad (1)$$

where A and B represent the *Y* intercepts of the distribution and elimination phases respectively, and alpha and beta representing slopes of the distribution and elimination phases on the natural logarithmic (ln) scale.

For modelling of the enterohepatic circulation, data were fitted to model PK40 as previously described[6]. The model is illustrated in Fig. 1 with the best fit equation below (2).

$$C(t) = \frac{In + K_a \times A_g - Cl \times C - Cl_d \times C_t - K_{1g} \times C \times V_c}{V_c}. \quad (2)$$

According to the equation, the plasma concentration at time t is dependent on the input (In), the absorption rate constant (K_a) from the gut into the central compartment, the amount of drug entering into the gut (A_g), plasma clearance (Cl), the diffusion parameter (Cl_d), concentration in the central compartment (C), concentration in peripheral compartment (C_t), bile excretion rate (K_{1g}), and volume of distribution of the central compartment (V_c). From this model, the following parameters were calculated: volume of the central compartment (V_c), plasma clearance (Cl), intercompartment diffusion (Cl_d), volume of the peripheral compartment (V_c), the absorptive rate constant (K_a), the bile excretion rate constant (K_{1g}) and the bile emptying interval (T_{tom}).

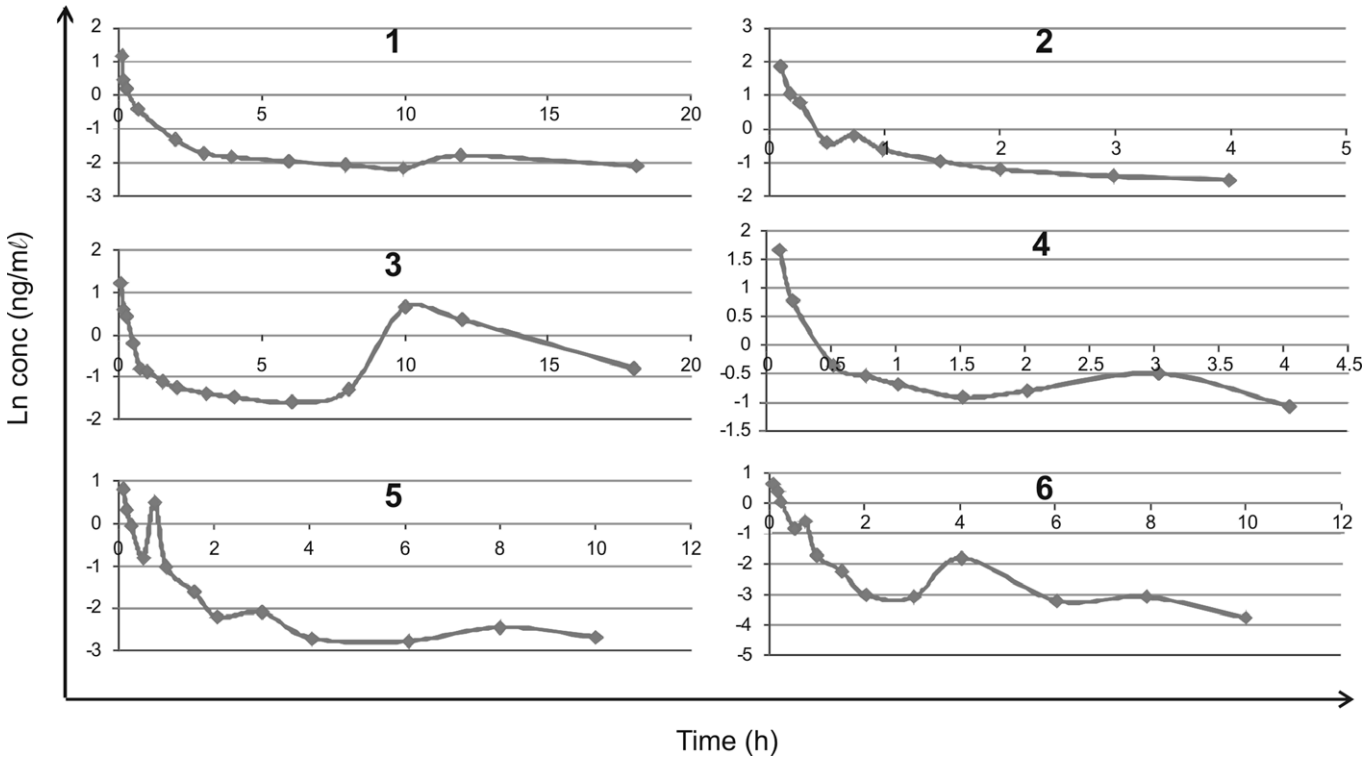

Fig 2: **The individual plasma *versus* time concentration profiles achieved for each animal following the intravenous administration of diminazene at 4.2 mg/kg.**

RESULTS

Two dogs (Nos 1 & 6) collapsed immediately after administration of the drugs with clinical signs of a weak erratic femoral pulse, irregular heartbeat, urination and vocalization. Both animals were fully recovered by 5 and 3 minutes, respectively from the initiation of clinical signs. No treatment was required for the affected animals.

Following the analysis of the plasma *versus* time profiles for the 6 dogs, none of the animals demonstrated consistent profiles (Fig. 2). Peaks were minor for animals 1 and 2 at 0.7 and 12 h, respectively, more pronounced for dogs 4 and 5 at 3 and 8 hours, respectively, and very pronounced in dogs 3 and 6 at 6 and 4 hours, respectively, and characteristically occurred towards the tail of the plasma profile (Fig. 2).

The non-compartmental parameters derived are presented in Table 1. Diminazene was characterised by a large volume of distribution of 2.28 ± 0.60 ℓ/h/kg and an apparent rapid rate of elimination of 2.83 ± 1.67 h from the central compartment. It had a mean residence time of 4.10 ± 1.58 h, AUC_{last} of 4.65 ± 1.08 μg/mℓ/h and plasma clearance of 0.77 ± 0.187 ℓ/h/kg.

When analysed by compartmental analysis, diminazene best fitted to the 2-compartment open model (Table 2). The 2-compartment model confirmed the high volume of distribution at 5.35 ± 1.517 ℓ/kg and clearance of 1.47 ± 0.317 ℓ/h/kg. Comparison of the distribution and elimination phases showed that diminazene underwent a rapid distributory phase with $T_{½}\alpha$ of 0.79 ± 0.707 h in addition to a rapid elimination phase of 4.03 ± 1.78 h. From the microconstants it appeared that the rate of movement of the drug into the peripheral compartment was faster than its rate of re-entry into the central compartment. A large degree of variability was once again evident between the treated animals.

In addition to a standard open 2-compartmental model, all profiles were fitted to the recirculatory enterohepatic model (Table 3). In contrast to the 2-compartmental model, diminazene was characterised by a small plasma clearance of

Table 1: **Pharmacokinetic parameters derived by non-compartmental modelling following intravenous administration to dogs at a dose of 4.2 mg/kg.**

Parameter	Dog						Mean	SE	Miller
	1	2	3	4	5	6			
C_0 (ng/mℓ)	1.88	9.17	3.61	4.16	2.81	2.56	4.03	1.08	
AUC_{last} (ng/mℓ/h)	3.91	2.95	14.21	3.34	2.03	1.48	4.65	1.95	5.09
AUC_{inf} (ng/mℓ/h)	4.26	3.18	14.42	3.91	2.12	1.53	4.90	1.95	6.06
L_z (/h)	0.15	0.59	0.15	0.44	0.32	0.29	0.32	0.07	0.07
$T_{½}$ (/h)	4.75	1.18	4.73	1.58	2.18	2.35	2.80	0.64	27.50
AUMC (ng/mℓ/h^2)	31.99	3.85	147.18	7.84	5.64	3.80	33.38	23.18	
MRT (h)	7.51	1.21	10.20	0.51	2.66	2.48	4.10	1.58	10.30
Cl (ℓ/h/kg)	0.47	0.63	0.14	1.16	0.94	1.30	0.77	0.18	0.83
V_z (ℓ/kg)	3.22	1.07	0.95	1.03	2.97	4.42	2.28	0.60	24.50
V_{ss} (ℓ/kg)	3.53	0.76	1.41	1.27	2.51	3.23	2.12	0.46	

C_0 = estimated concentration at 0 h, AUC_{last} = area under curve to the last sample point, AUC_{inf} = area under curve extrapolated to infinite, L_z = elimination constant, $T_{½}$ = half-life of elimination, AUMC = area under the moment curve, MRT = mean residence time, Cl = clearance, V_z = volume of distribution, V_{ss} = volume of distribution at steady state. Miller = mean values of Miller *et al.*[11].

Table 2: **Pharmacokinetic parameters derived by 2-compartmental modelling following intravenous administration to dogs at a dose of 4.2 mg/kg.**

Parameter	1	2	3	4	5	6	Mean	SEM	Miller
A (ng/mℓ)	3.84	15.74	4.80	13.98	11.82	0.15	8.39	2.57	7.38
α (/h)	3.38	10.89	6.25	11.07	24.31	0.16	9.34	3.46	2.30
B (ng/mℓ)	0.18	1.07	0.55	0.68	1.30	2.29	1.01	0.30	0.53
β (/h)	0.08	0.58	0.23	0.07	0.80	3.23	0.83	0.49	0.16
AUC_{last} (ng/mℓ/h)	3.36	3.29	3.09	11.49	2.10	1.62	4.16	1.49	
AUMC (ng/mℓ/h^2)	27.40	3.34	10.01	155.03	2.03	5.84	33.94	24.51	
MRT (/h)	8.16	1.01	3.24	13.49	0.96	3.60	5.08	1.99	
L_z (/h)	0.08	0.58	0.23	0.07	0.80	0.16	0.32	0.12	
K_{10} (/h)	1.20	5.10	1.73	1.28	6.24	1.51	2.84	0.91	0.11
K_{12} (/h)	2.03	5.14	3.91	9.29	15.74	1.54	6.27	2.21	1.13
K_{21} (/h)	0.23	1.23	0.85	0.57	3.14	0.35	1.06	0.44	0.52
$T_{½\alpha}$ (h)	0.21	0.06	0.11	0.06	0.03	4.27	0.79	0.70	0.36
$T_{½\beta}$ (h)	8.45	1.20	2.95	10.50	0.86	0.21	4.03	1.78	5.31
$T_{½Lz}$ (h)	8.45	1.20	2.95	10.50	0.86	4.27	4.71	1.61	
V_{ss} (ℓ/kg)	10.22	1.29	4.40	4.93	1.93	9.33	5.35	1.51	
V_z (ℓ/kg)	15.26	2.21	5.79	5.54	2.48	15.98	7.88	2.52	
Cl (ℓ/h/kg)	1.25	1.28	1.36	0.37	2.00	2.59	1.47	0.31	

A = intercept of the distribution phase, α = distribution constant, B = intercept of the elimination phase, β = elimination constant, AUC_{last} = area under curve to the last sample point, AUMC = area under the moment curve, MRT = mean residence time, L_z = biological half-life constant, K_{10}, K_{12}, K_{21} = model rate constants, $T_{½\alpha}$ = half-life of distribution, $T_{½\beta}$ = half-life of elimination, $T_{½lz}$ = biological half-life, V_{ss} = volume of distribution at steady state, V_z = volume of distribution, Cl = clearance. Miller = mean values of Miller *et al.*[11].

2.44 ± 1.49 mℓ/h/kg. The clearance from the peripheral compartment was also low, although much more rapid at 25.86 ± 11.06 mℓ/h/kg than the 2-compartmental open model. The overall volume of distribution of the drug was very small 126.34 ± 89.08 mℓ/kg, which was markedly different from the volume of the peripheral compartment at 1.847 ℓ/kg/h. The rate of release of the diminazene from the gall bladder was slow at 58.96 ± 51.167 h, with T_{tom} also large at 277 h for all animals, with animal 2 having a largest value of 129.99 h. Once released into the GIT, reabsorption was also slow at 9.70 ± 5.48 h.

DISCUSSION

The collapse observed in the 2 dogs following intravenous administration has been previously described[2]. While the exact cause of the adverse drug reaction is unknown, it most likely resulted from the choline-esterase inhibitory properties of diminazene as the signs were typically cholinergic[12]. This was, however, contrary to the findings of Milner *et al.*[12] and Joubert *et al.*[9], who demonstrated no significant changes in the overall plasma choline-esterase activity and systemic blood pressure, respectively, when the drug was administered intramuscularly. The adverse reaction observed following administration is most likely related to the rapid administration of the product intravenously, which would not occur following intramuscular administration owing to the process of absorption.

Initially the investigation was designed to demonstrate that the secondary peak seen by Miller *et al.*,[11] was related to the route of administration as opposed to redistributory pharmacokinetics of diminazene. The presence of the secondary peak, following intravenous administration, illustrates that these peaks are related to the redistribution of the molecule. For this reason the pharmacokinetic data were fitted to an enterohepatic recirculatory model. The delay in the occurrence of the secondary peak was due to the slow release of the drug for the absorptive phase, which normally would be related to a delay in the release of bile from the gall bladder. However, due to the very large T_{tom} value of 277 h, it is unlikely that the delay in absorption phase results from the gall bladder, as the normal release of bile occurs in cycles of 27 h. This therefore suggests that billiary excretion *via* an enterohepatic circulation is not the cause of the prolonged mean residence time of the drug but rather that the drug was sequestered and released elsewhere in the body. Considering that the kidney and liver showed the highest concentration in the study by Onyeyili and Anika[16], it is suggested that the drug distributes to these organs and is released

Table 3: **Pharmacokinetic parameters derived from the enterohepatic circulation model following intravenous administration to dogs at a dose of 4.2 mg/kg.**

Parameter	Dog						Mean	SEM
	1.00	2.00	3.00	4.00	5.00	6.00		
V_c (mℓ/kg)	20.15	3.02	28.20	34.32	106.59	565.77	126.34	89.08
Cl (mℓ/h/kg)	0.01	7.45	0.12	6.87	0.00	0.22	2.44	1.49
Cl_d (mℓ/kg)	15.47	0.76	8.07	28.51	25.40	76.94	25.86	11.06
V_t (ℓ/kg)	12.59	0.02	8.44	2.06	4.13	32.15	9.90	4.82
K_a (/h)	0.69	122.95	0.02	0.40	0.23	0.03	20.72	20.45
k_{1g} (/h)	0.07	0.00	0.10	0.47	0.11	0.05	0.13	0.07
T_{tom} (h)	2.43	129.99	26.64	2.06	3.22	0.43	27.46	20.90
K_a-HL (h)	1.01	0.01	32.41	1.71	3.00	20.03	9.70	5.48
K_{1g}-HL (h)	10.11	314.63	6.92	1.48	6.25	14.35	58.96	51.16

V_c = volume of distribution of the central compartment, Cl = clearance, Cl_d = intercompartmental clearance, V_t = volume of the peripheral compartment, K_a = absorptive rate constant, Kig-Bile excretion rate, T_{tom} = bile emptying interval.

from these tissues over time, perhaps even to nucleic acids as seen with imidocarb. Also evident from the model was the slow half-life of the release of drug from the area of binding and its subsequent re-entry into the central compartment. If the assumption of the binding of the drug to hepatic and/or renal issue and its subsequent release is correct, then the absorptive phase is actually a delayed redistributory phase.

In comparison with the intramuscular profiles of Miller *et al.*[11], the secondary peak occurred much earlier for the i.v. administered drug in comparison with the ±1207 h for i.m. administration. More interestingly in comparison to the study of Anika and Onyeyili[1], the animals in this study demonstrated secondary peaks, while they were absent in the that study. Perhaps if the profile had been followed for a longer period or at more frequent time intervals, more peaks would have been identified during the cycle. Major differences were also present between the compartmental and 2-compartmental models of Miller *et al.*[11], which would once again support the assertion that the pharmacokinetics of diminazene in dogs are extremely variable. This would to a large extent explain the unpredictable nature of toxicity, *i.e.* why some dogs are poisoned at the normal therapeutic dose[5].

CONCLUSIONS

From the individual pharmacokinetic parameters determined, diminazene was best analysed by a compartment recirculatory enterohepatic open model when all time points are taken into account. While the model is specific to enterohepatic recirculation, the long delay in the release of the drug from its area of sequestration tends to suggest that the gall bladder is not the area of sequestration. For this to be confirmed, radio-tagged studies with a detailed analysis of the biliary excretory profile needs to be undertaken to elucidate the full pharmacokinetics of diminazene in dogs.

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