The pharmacokinetics of diminazene aceturate after intramuscular administration in healthy dogs

D M Miller[a], G E Swan[b*], R G Lobetti[a] and L S Jacobson[a]

ABSTRACT

The pharmacokinetics of diminazene aceturate following intramuscular (i.m.) administration at 4.2 mg/kg was evaluated in 8 healthy German Shepherd dogs. Blood samples were collected at 19 intervals over a period of 21 days. Diminazene plasma concentrations were measured using a validated HPLC method with UV detection and a sensitivity of 25 ng/mℓ. The *in vitro* and *in vivo* binding of diminazene to blood elements was additionally determined. Diminazene pharmacokinetics showed a large inter-individual variation after i.m. administration. It had a short absorption half-life (K01-HL of 0.11 ± 0.18 h), resulting in a C_{max} of 1849 ± 268.7 ng/mℓ at T_{max} of 0.37 h and a mean overall elimination half-life ($T_{½\beta}$) of 5.31 ± 3.89 h. A terminal half-life of 27.5 ± 25.0 h was measured. At 1 h after i.m. injection, 75 % of the diminazene in whole blood was in the plasma fraction. The results of this study indicate that diminazene is rapidly distributed and sequestered into the liver, followed by a slower terminal phase during which diminazene is both redistributed to the peripheral tissues and/or renally excreted. It is recommended that diminazene administered i.m. at 4.2 mg/kg should not be repeated within a 21-day period.

Key words: *Babesia canis*, babesiosis, berenil, canine, diminazene, liver sequestration, pharmacokinetics, pharmacology.

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INTRODUCTION

Diminazene is the antibabesial drug of choice for the treatment of canine babesiosis in South Africa[5]. Differences in the dosage described for diminazene and the occurrence of mortality at doses equal to or close to the recommended therapeutic dose for the treatment of canine babesiosis have been described[14,17,18,22,25]. The diminazene products used to treat babesiosis in South Africa contain a combination of diminazene aceturate and antipyrine, mostly in a concentration of 45 % m/m and 55 % m/m, respectively.

Babesia canis is a tick-transmitted intraerythrocytic protozoan parasite responsible for high morbidity and mortality among dogs in South Africa[23]. The incidence of canine babesiosis at the outpatients clinic of the Onderstepoort Veterinary Academic Hospital over a 6-year period was 11.7 % (1253 of 10710 sick dogs presented per year)[24]. In 1955, Fussgänger wrote of his work on the antibabesial drug diminazene: 'Remarkable therapeutic success has been obtained during the last years in the treatment of protozoal diseases in domestic animals using a novel drug developed in the research laboratories of Fabwerke Hoechst A.G'[7]. However, little pharmacokinetic work with diminazene aceturate has since been done in dogs.

Bauer[3] reported peak serum concentrations of diminazene (3 μg/mℓ) occurred at 3 hours (h) after i.m. administration in the dog and that all traces of diminazene were absent by 24 h. He concluded that diminazene was excreted *via* the kidneys within 24 h. Onyeyili and Anika[20,21] looked at the influence of *Trypanosoma congolense* on the disposition of diminazene in the dog and reported that drug elimination followed a biphasic process. This was seen irrespective of infection, but infection significantly shortened the half-life of absorption $T_{½\alpha}$ of diminazene from 0.17 h to 0.12 h, although the urinary excretion of the drug remained constant[20]. That study used 3.5 mg/kg diminazene intramuscularly (i.m.) in both healthy dogs and in dogs with trypanosomiasis. Mean plasma concentrations were 0.2 ± 0.008 μg/mℓ, but no peak concentrations were reported. No diminazene was found in the plasma after 48 h. Higher plasma concentrations were found in dogs infected with trypanosomes, and higher tissue concentrations were present in healthy dogs. In tissues sampled at 48 h, the highest concentrations of diminazene were found in the kidneys and liver in both groups and low diminazene concentrations were found in the brain. Diminazene persisted in the tissues for more than 10 days[21]. The same authors subsequently reported a biological half-life ($T_{½\beta}$) of 9.87 h in healthy dogs and 12.51 h. in *T.b. brucei*-infected dogs. The $T_{½\alpha}$ was significantly decreased after trypanosome infection (0.14 h *vs* 0.2 h)[20].

Healthy dogs given i.m. diminazene showed severe clinical signs associated with damage to the central nervous system (CNS) and then died[18]. Interestingly, 1 dog was resistant to the toxic effects of diminazene despite repeated daily i.m. treatments at 3.5 mg/kg for 15 and 30 doses, while other dogs showed typical neurological clinical signs after only 2 doses. At necropsy the brains were oedematous and showed bilaterally symmetrical haemorrhages together with malacic lesions of the cerebellum, midbrain and thalamus[18]. Healthy dogs treated with 15 mg/kg of diminazene i.m. had brain lesions mimicking those of cerebral babesiosis[14]. The incidence of CNS toxicity is not known but it has been reported to occur both with overdose and at the recommended dose[14,18,22].

Alvi *et al.*[1] incubated diminazene with blood products and found that plasma and serum binding was 50 % and 35 %, respectively, with 70 % of diminazene bound to purified haemoglobin, whereas red blood cell membranes did not show any binding. They concluded that diminazene binds to a number of blood proteins and could cross the red cell membrane to bind to haemoglobin.

The unpredictability of diminazene toxicity, with mortalities reported at doses equal to or close to the recommended therapeutic dose for the treatment of canine babesiosis[14,18,22], dictated the need

[a]Department of Companion Animal Studies and [b]Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

*Author for correspondence.
E-mail: gerry.swan@up.ac.za

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for further study. A better understanding the pharmacokinetics and macromolecular binding features of diminazene in dogs is needed to explain the cause for the variation in the clinical responses seen. The current study was undertaken to examine the pharmacokinetics of diminazene aceturate after intramuscular injection in healthy adult dogs.

MATERIALS AND METHODS

Animals

The Animal Use and Care Committee of the Faculty of Veterinary Science approved this study (Study No. 36.5.274).

Eight male German Shepherd dogs, of approximately the same age (18 months) and weight range (30 ± 2.5 kg) were used. The dogs were obtained from the Roodeplaat dog breeding station, of the South African Police Service. Once the study was completed, the dogs were returned to the breeding station, to continue their normal duties.

Dogs were included in this prospective study if they were fully vaccinated, were found to be clinically healthy and were easy to handle. Dogs that had been treated for canine babesiosis in the preceding 3 months, received any medical therapy for any disorder within a 3-week period, or had been dipped for ectoparasite control within a 2-week period prior to the start of the study, were excluded.

Full clinical examination, urine analysis, complete haematology and serum biochemistry (total serum protein, albumin, globulin, alkaline phosphatase, alanine aminotransferase, urea and creatinine) were performed to rule out any underlying disease that might affect diminazene pharmacokinetics. All the test results were within normal limits.

Treatment

Multiple bottles of diminazene (Berenil, Hoechst Roussel Vet, Halfway House, South Africa), from the same batch, were repackaged by Kyron laboratories (Kyron Laboratories, Benrose, South Africa) specifically for the study. The bottles were weighed to ensure that they contained the correct amount of content. A sample of the contents of each bottle was kept to analyse the drug concentration after dilution. The mixture was prepared by reconstituting the 1.05 g diminazene with 25 mℓ sterile water to give a resultant volume of 25 mℓ and a concentration of 42 mg/mℓ.

All the dogs were weighed 2 days prior to the start of the study, following a 12-h period of food withdrawal and after the dogs had been taken out to urinate and defaecate. The dose of diminazene for each dog was calculated and recorded according to its fasted body weight.

The dogs were injected with freshly diluted diminazene at the standard dose of 4.2 mg/kg. Feed was withdrawn from all the dogs 12 h before to 4 h after treatment. Intramuscular injections were performed in the *M. biceps femoris,* midway between the hip and the stifle joints.

Blood collections

Five mℓ blood samples were collected 1–2 min pre-treatment (Time 0), and at 0.33, 0.66, 1, 2, 3, 4, 8, 12, 18, 24, 36, 48, 72, 120, 168, 240, 336 and 504 h post-treatment. The sample timing was based on available diminazene pharmacokinetic data, with specific attention given to the peak concentration (C_{max}) and overall elimination half-life ($T½\beta$), reported in the literature. Blood was drawn into evacuated, uniquely identified heparinized tubes (Vacutainer, BD Vacutainer Systems, Preanalytical Solutions, Belliver, Plymouth, UK) from either the cephalic or jugular veins. The heparinized blood was stored on ice until centrifuged. Samples were centrifuged within 30 min of collection at 3000 r.p.m for 15 min, the plasma transferred to polycarbonate tubes and stored at –20°C until analysed.

Diminazene binding to blood elements

The binding of diminazene to plasma and red blood cell contents was examined both *in vitro* and on *ex vivo* samples. For the *in vitro* study 425 mℓ of blood, collected in acid-citrate dextrose was drawn from a dog chosen following the same selection criteria as used for the dogs in the pharmacokinetic study. Three 50 mℓ bags of the blood were fortified to 3 different concentrations of diminazene, 0.5, 1.5 and 3 μg/mℓ. The blood bags were placed in a refrigerator at 4°C for 24 h to allow drug to red blood cell binding to occur. They were turned every 4 h to ensure mixing of the diminazene and blood.

After 24 h, two 10 mℓ samples of blood was withdrawn from each bag, centrifuged and divided into plasma and packed RBC. The packed RBC from each individual bag was washed 3 times using physiological saline and then pooled. Half of the plasma was microcentrifuged through a 10 000 micropore filter at 10 000 rpm for 30 min (Beckman CS-15R centrifuge with Beckman F1010 head, radius 8 cm, Beckman Coulter, Midrand, South Africa). This procedure left 3 samples of (1) washed packed RBC; (2) plasma and (3) filtered plasma (water fraction). All samples for the *in vitro* study were processed on the same day and 6 replicates were collected for each fraction. These samples also acted as the *in vitro* quality control to test for the repeatability of the analyses.

Additional 5 mℓ heparinised sample were collected from all trial animals at 1 h after treatment for *ex vivo* analysis. These were separated into fractions as described above and pooled. The pooled fractions were then split into as many 1 mℓ aliquots as the available volume allowed. Five replicates of each pooled fraction were analysed and the average concentration for each fraction determined.

Diminazene assay

Diminazene concentrations were determined by a validated high performance liquid chromatographic (HPLC) method with UV detection following paired ion extraction[10]. The method had a limit of quantification of 25 ng/mℓ.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by non-linear compartmental analysis and non-compartmental analysis for extravascular administration of the diminazene plasma concentration *versus* time data. The analysis was performed by means of PC Nonlin Version 4.2 (Statistical Consultants, Inc., New York, USA) using the Nelder-Mead algorithm. Initial pharmacokinetic parameter estimates, used for the non-linear analysis, were derived automatically by initial linear analysis performed by the program. Akaike's information criterion[28], based on the mean values of the final estimates of the associated pharmacokinetic parameters and lack of systematic deviations around the fitted disposition curve, was used to determine the number of exponential terms that best described the data.

Primary pharmacokinetic parameters were derived by 2-compartmental analysis with 1st-order input, 1st-order output and lag time (Model 13) of the plasma concentration-time data for each individual animal, yielding the microconstants K01 and K10. Secondary disposition parameters, including area under the curve (AUC), K01 half-life (K01-HL), K10 half-life (K10-HL), overall absorption and distribution half-life ($T½\alpha$) and overall elimination half-life ($T½\beta$), were derived from the primary parameters utilising standard procedures[8].

The area under the plasma concentration *versus* time curve (AUC, zero-moment) and the 1st non-normalised moment (AUMC) were calculated according to the trapezoidal method from time zero to the last sample time as derived by non-compartmental analysis. Extrapolation of AUC to infinity (AUC_{inf}) was performed using the slope of the terminal phase (β). Since AUMC to infinity could not be

determined in some animals these were not reported. The mean residence time (MRT, 1st moment) was derived from AUC/AUMC and terminal half-life (T½λ) by linear regression of the terminal diminazene plasma concentration *versus* time points (n = 4). Maximum plasma concentration (C_{max}) of diminazene and time to C_{max} (T_{max}) were read directly from the individual plasma concentrations.

The pharmacokinetic analysis was truncated at the 72 h sample as diminazene plasma concentrations of 6 of the 8 dogs were below the level of detection at this time point and the remaining 2 dogs had very low levels that fluctuated widely.

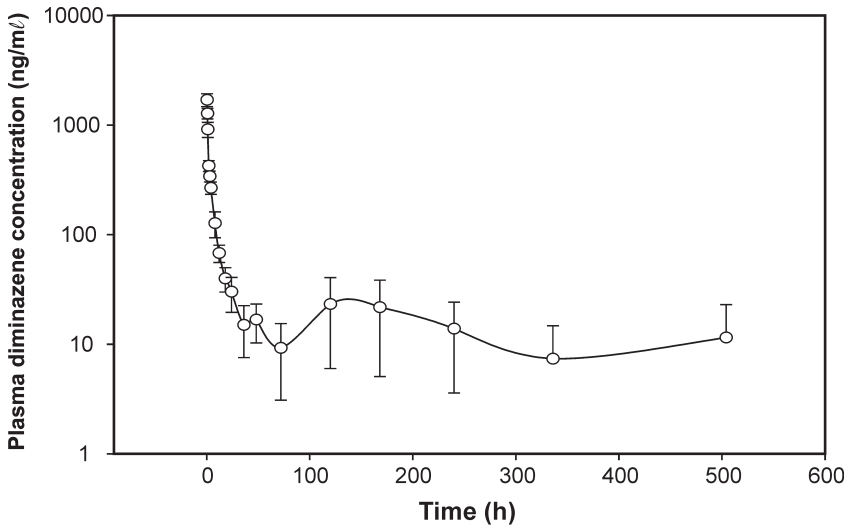

Fig. 1: **Natural logarithm of diminazene plasma concentration** (mean ± SD) ***versus*** **time profile in dogs** (n = 8) **following intramuscular administration of diminazene aceturate at 4.2 mg/kg.**

RESULTS

None of the dogs showed any pain reaction at the time of injection and no muscle swelling was seen at the injection site. Three dogs developed diarrhoea within 20 min of diminazene administration. One of these dogs had diarrhoea again after 45 min while 2 other dogs developed diarrhoea after 1 h and after 1 h 15 min. All the dogs in the trial ate (when they were offered food) after the 4 h sample had been drawn and none of the dogs exhibited diarrhoea again for the duration of the study.

A semilogarithmic plot of mean diminazene concentrations *versus* time was constructed (Fig. 1). The derived compartmental and non-compartmental data are summarised in Tables 1 and 2. Compartmental pharmacokinetic analysis revealed a very rapid rate of absorption as measured by the half-life of absorption (K01-HL) 0.11 ± 0.18 h and rapid overall absorption and distribution half-life (T½α) 0.36 ± 0.19 h (Table 1). The distribution into the peripheral compartment was more rapid than the distribution back into the central compartment. A mean overall elimination half-life (T½β) of 5.31 ± 3.89 h was derived. There was large inter-subject variation in the pharmacokinetic results (% CV range of 37–163 for the various pharmacokinetic parameters).

Peak plasma concentrations (C_{max}) of 1849.9 ± 268.7 ng/mℓ occurred at 0.37 ± 0.12 h (T_{max}) after intramuscular administration. A terminal half-life of 27.5 ± 24.96 h and MRT of 10.32 ± 5.44 h were observed following non-compartmental analysis.

The binding of diminazene to blood elements (Table 3) revealed that the drug was predominantly bound to plasma. Seventy-five per cent of diminazene in whole blood was present in the plasma 1 h after i.m. injection while 24 h after the *in vitro* blood/diminazene admixture, 85–94.5 % of the diminazene was extracted from the plasma fraction. The mean percentage of diminazene in the filtrate (water fraction) was 17 % of the total plasma diminazene, for the 3 concentrations of diminazene in the *in vitro* study and 24 % for *ex vivo* 1 h samples. The plasma concentration of diminazene from the *in vivo* study, was corrected for a haematocrit of 50 % so that the equations reflected the actual volume of plasma per mℓ of blood rather than the diminazene concentrations per mℓ of plasma. Seventy-six per cent of the diminazene was extracted from the plasma fraction and the percentage of diminazene bound to the red blood cells was 18.5 % of the total diminazene in the blood.

DISCUSSION

This is the first comprehensive study of the pharmacokinetics of diminazene following i.m. administration in dogs.

Table 1: **Pharmacokinetic results following intramuscular administration of diminazene aceturate at 4.2 mg/kg in dogs** (n = 8) **derived by 2-compartmental analysis.**

Variable	Individual dog results									
	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	Dog 7	Dog 8	Mean ± SD	% CV
A (ng/mℓ)	25063	3156	4219	2646	2435	2479	15315	3718	7379 ± 83478	113.1
B (ng/mℓ)	393	546	566	525	709	802	144	556	530 ± 199	37.5
K01	3.50	5.21	89.34	50.00	50.01	67.97	1.31	75.91	42.9 ± 35.2	82.0
α (h^{-1})	2.87	1.00	3.03	1.76	2.76	2.59	1.13	3.27	2.30 ± 0.88	38.4
β (h^{-1})	0.0840	0.0926	0.1958	0.2019	0.0283	0.3711	0.0536	0.2740	0.163 ± 0.119	73.3
K10	0.80	0.37	1.10	0.76	0.90	1.37	0.50	1.32	0.89 ± 0.36	40.5
K12	1.85	0.47	1.59	0.73	1.28	1.00	0.56	1.55	1.13 ± 0.52	46.0
K21	0.3015	0.2502	0.5410	0.4658	0.8638	0.9283	0.1210	0.6784	0.519 ± 0.291	56.1
K10-HL (h)	0.87	1.87	0.63	0.91	0.77	0.67	1.38	0.52	0.95 ± 0.45	47.4
K01-HL (h)	0.20	0.13	0.01	0.01	0.01	0.01	0.53	0.01	0.11 ± 0.18	163.6
T½α (h)	0.24	0.69	0.23	0.40	0.25	0.27	0.61	0.21	0.36 ± 0.19	52.8
T½β (h)	8.25	7.49	3.54	3.43	2.45	1.87	12.92	2.53	5.31 ± 3.89	73.3

A, distribution phase intercept (initial serum drug concentration); B, elimination phase intercept; K01, rate at which drug enters the central compartment; α, overall absorption and distribution constant; β, overall elimination constant; K10, rate at which drug leaves the central compartment; K12, rate constant for drug removal/distribution from central compartment; K21, rate constant for distribution from peripheral compartment; K10-HL, half life of K10; K01-HL, half-life of K01; T½ α, overall absorption and distribution half-life; T½β, overall elimination half-life.

Table 2: **Pharmacokinetic results following intramuscular administration of diminazene aceturate at 4.2 mg/kg in dogs** (n = 8) **derived by non-compartmental analysis.**

Variable	Individual dog results									
	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	Dog 7	Dog 8	Mean ± SD	% CV
T_{max} (h)	0.33	0.33	0.33	0.33	0.33	0.33	0.66	0.33	0.37 ± 0.12	31.42
C_{max} (ng/mℓ)	1998	2188	2083	1983	1632	1779	1361	1775	1850 ± 269	14.52
K_λ	0.0574	0.0165	0.0224	0.0139	0.0098	0.1854	0.1388	0.1196	0.071 ± 0.068	96.73
T½λ (h)	12.07	41.93	31.01	49.77	70.69	3.74	4.99	5.80	27.5 ± 25.0	90.76
AUC_{last} (ng/h/mℓ)	6283	9746	4935	4996	4929	3041	3769	3013	5089 ± 2184	42.91
AUC_{inf} (ng/h/mℓ)	6510	12348	5517	5930	8091	3111	3863	3122	6062 ± 3078	50.78
Vc/f (ℓ/kg)	11.2	20.6	34.1	50.9	52.9	7.3	7.8	11.2	24.5 ± 19.1	77.76
Cl/f (mℓ/kg/h)	0.6	0.3	0.8	0.7	0.5	1.3	1.1	1.3	0.83 ± 0.37	45.13
MRT (h)	10.01	14.03	14.06	13.37	18.06	3.21	5.42	4.38	10.3 ± 5.4	52.71

Tmax, time to peak plasma concentrations; Cmax, maximum plasma concentrations; K_λ, 1st-order rate constant associated with the terminal (log-linear) portion of the curve; T½λ, terminal half-life; AUC_{last}, area under the plasma concentration curve to the last measurable plasma concentration; AUC_{inf} extrapolated to infinity; Vc/f, fractional volume of distribution of the central compartment; Cl/f, fractional total body clearance; MRT, mean residence time.

Table 3: **Macromolecular binding of diminazene in canine blood examined *in vitro* and *in vivo*.**

Sample	Diminazene concentrations (ng/mℓ)			Binding (%)	
	Plasma	Filtrate	RBC	Plasma	RBC
In vitro					
500 ng/mℓ	330	69	48	85	4.8
1500 ng/mℓ	1152	127	49	94.5	1.6
3000 ng/mℓ	2138	410	148	93.1	4.4
In vivo					
1 h sample*	913 ± 408**	222**	339**	75.7*	18.5*

*Mean of all dogs. **Mean of 2 animals.

The pharmacokinetics of diminazene were characterised by a very rapid rate of absorption and short overall elimination half-life (T½β, 5.31 ± 3.89 h). The maximum plasma concentrations (C_{max}: 1850 ± 269 ng/mℓ) measured at 22.2 ± 6 min (T_{max}) were most likely an underestimation since the peak plasma concentrations were already measured in the 1st blood samples collected, at 20 min after treatment, in 7 of the 8 dogs.

The precipitous drop in plasma concentrations was ascribed to rapid distribution of diminazene into the peripheral compartment. It seems likely that the liver serves as an initial sump for diminazene. Onyeyili and Anika[20] found that 7 kg dogs, given 3.5 mg/kg of diminazene, had 81 μg/g of diminazene in their livers 48 h after diminazene administration. This accounts for 78.8 % of the total drug if one takes the liver weight at 3.4 % of body weight[9].

Gummow found that injection site reactions occurred in cattle[11] and reasoned that this could result in secondary peaks. It is possible that the same reaction is responsible for the biphasic absorption seen in this current study, but as the distribution phase is faster than renal excretion (normal creatinine clearance rates 2.8 ± 0.96 mℓ/min/kg to 4.09 ± 0.52 mℓ/min/kg[6,23]) sequestration within the liver is the more likely hypothesis. This would also explain the differences in the rate of distribution to and from the peripheral compartment (K12 *versus* K21).

Higher plasma concentrations of diminazene were found in dogs infected with trypanosomes while higher tissue concentrations were present in healthy dogs[2]. The total body clearance was significantly lower in healthy dogs and the distribution half-life was significantly decreased in infected dogs[2]. Onyeyili *et al.*[20] found significantly higher diminazene residues in the tissues of healthy dogs than in dogs infected with *T. congolense* in all tissues tested except in the brain. The distribution half-life was significantly reduced after infection and infection increased the rate at which diminazene was distributed to the body[19–21]. Mamman *et al.*[15], in a study of healthy cattle and those with acute and chronic *T. congolense* infection, found that C_{max} was significantly greater and T_{max} significantly shorter in the acute infection group. Similar studies have, however, not been reported in *Babesia*-infected dogs or cattle.

In dogs with babesiosis, factors such as hypotension, anaemia, acidosis, changes in albumin concentrations and altered endothelial integrity[4,27], as well as possible altered drug absorption from the injection site may alter the pharmacokinetics of diminazene. Alterations in hepatic and renal perfusion may have a large influence on diminazene distribution and thus may influence plasma concentrations of diminazene and play a role in either potentiating or decreasing the chances of toxicity. Furthermore a definite diagnosis of diminazene toxicity is complicated by the difficulty in distinguishing this from cerebral babesiosis, as well as from the clinical signs of hypoglycaemia in severe babesiosis[13].

The CNS toxicity of diminazene is dose-related, and repeated doses of diminazene can also cause CNS toxicity due to cumulative effects. The current study demonstrates that diminazene is extensively distributed, but it is not known how this translates into tissue concentrations within the brain or what effect it may have on transport mechanisms in the blood–brain barrier[16,26]. From the study results, a washout time of 1–5 days (5 half-lives), based on T½β and T½λ, respectively, would appear to be sufficient. Despite this, a washout period of at least 21 days is recommended. This conservative approach is suggested by the finding that most of the drug is distributed to the peripheral compartment and that the limit of detection was not low enough to determine the terminal phase and the AUC correctly. In one canine study, diminazene persisted in the tissues for over 10 days[21]. In addition, the babesiosis disease process may alter diminazene pharmacokinetics, as alluded to above, and due to the fact that bile acid levels were raised in a third of severely ill animals with babesiosis[27]. Hypotension is a common phenomenon in clinical babesiosis[12] and is usually associated with splanchnic [and thus hepatic] vasoconstriction to ensure cerebral blood flow. Apparent differences in the susceptibility of dogs to diminazene toxicity have been implied in South Africa and this is sup-

ported by the fact that some dogs were resistant to 20 mg/kg diminazene i.m. repeatedly, while other dogs showed signs of diminazene toxicity at the recommended dosage[14,18,22]. The role of the blood–brain barrier extrusion pumps in the occurrence of ivermectin toxicity has been implicated in certain breeds[16], raising the question of whether this may also be applicable to diminazene toxicity. These factors are of significance taking into consideration the distribution pharmacokinetics of diminazene, in particular the role of the liver and the increased distribution of diminazene to the brain in *Trypanasoma*-infected dogs[20]. A lower dose might be considered for dogs with compromised liver function, which could be at higher risk for toxicity.

Collapse, salivation and diarrhoea have been described in clinically healthy dogs given large doses of diminazene i.v.[18] but have not previously been reported following i.m. administration, and diarrhoea following diminazene administration in dogs with babesiosis might erroneously be ascribed to the disease. The fact that 5 of the 8 dogs in this study displayed bouts of diarrhoea after i.m. diminazene administration is worth taking note of.

Alvi *et al.*[1] reported that diminazene bound to a number of blood proteins and could cross the red cell membrane to bind to haemoglobin. This was refuted by the current study. The binding of diminazene to red blood cells is markedly less than plasma, and is not expected to play an important role in the $T_{1/2}\beta$. Therefore no dose adjustments should be necessary for anaemic patients.

CONCLUSION

Diminazene is most likely first sequestered in the liver, then is slowly released back into the central compartment and redistributed into less well perfused peripheral tissues before finally being eliminated. The slower redistribution to and from these peripheral tissues is likely to be mainly responsible for the longer elimination half-life of diminazene as measured by non-compartmental analysis. An apparent low bioavailablility could be explained by retention of a portion of the dose at the site of injection as was seen in cattle and to the fact that the C_{max} might have been missed in some of the dogs which could also have resulted in a smaller AUC measured after i.m. administration.

Since the terminal portion of the diminazene plasma concentration *versus* time curve of the i.m. data could not be clearly defined it is advise that a longer withdrawal time and that care should be taken in dogs with decreased liver mass. With the knowledge gained of the pharmacokinetics of diminazene in healthy dogs, a population pharmacokinetic study in dogs with babesiosis is recommended. This will allow us to more fully appreciate alterations in pharmacokinetics of diminazene and the potential covariants playing a role. A study of this nature would help to elucidate if there is any therapeutic implication due to large inter-individual variation. This may lead to the possible contributing causes of diminazene toxicity.

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REFERENCES

1. Alvi N K, Haqqi T M, Hadi S M 1985 Irreversible binding of Berenil, a trypanocidal drug to blood proteins. *Indian Journal of Experimental Biology* 23: 172–173
2. Anika S M, Onyeyili P A 1989 Effects of trypanosomal infections on the pharmacokinetics of diminazene aceturate in dogs. *Tropical Medicine and Parasitology* 40: 419–421
3. Bauer F R, Ergebnisse der klinischen Prufung von Berenil. Hoechst, Germany
4. Brady C A, Otto C M 2001 Systemic inflammatory response syndrome, sepsis and multiple organ dysfunction. *Veterinary Clinics of North America Small Animal Practice* 31: 1147–62
5. Collett M G 2000 Survey of canine babesiosis in South Africa. *Journal of the South African Veterinary Association* 71: 180–186
6. Finco D R, Tabaru H, Brown S A, Barsanti J A 1993 Endogenous creatinine clearance measurement of glomerular filtration rate in dogs. *American Journal of Veterinary Research* 54: 1575–1578
7. Fussgänger R, 1995. Berenil in veterinary medicine: report from the chemotherapeutical Institute of Faberwerke Hoechst, AG, Germany
8. Gibaldi M, Perrier D 1982 *Pharmacokinetics.* Marcel Dekker, New York
9. Guilford W G, Center S A, Strombeck D R, Williams D A, Meyer D J (eds) 1996 *Strombeck's small animal gastroenterology* (3rd edn). W B Saunders, Philadelphia
10. Gummow B, Du Preez J L, Swan G E 1995 Paired-ion extraction and high-performance liquid chromatographic determination of diminazene in cattle plasma: a modified method. *Onderstepoort Journal of Veterinary Research* 62: 1–4
11. Gummow B, Swan G E, Du Preez J L 1994 A bioequivalence and pharmacokinetic evaluation of two commercial diminazene aceturate formulations administered intramuscularly to cattle. *Onderstepoort Journal of Veterinary Research* 61: 317–326
12. Jacobson L S, Lobetti R G, Vaughan-Scott T 2000 Blood pressure changes in dogs with babesiosis. *Journal of the South African Veterinary Association* 71: 14–20
13. Keller N, Jacobson L S, Nel M, de Clerq M, Thompson P N, Schoeman J P 2004 Prevalence and risk factors of hypoglycaemia in virulent babesiosis. *Journal of Veterinary Internal Medicine* 18: 265–270
14. Losos G J, Crockett E 1969 Toxicity of berenil in the dog. *Veterinary Record* 85: 196
15. Mamman M, Aliu Y O, Peregrine A S 1993 Comparative pharmacokinetics of diminazene in noninfected boran (*Bos indicus*) cattle and boran cattle infected with *Trypanosoma congolense. Antimicrobial Agents and Chemotherapy* 37: 1050–1055
16. Mealey K L, Bentjen S A, Gray J M, Cantor G H 2001 Ivermectin sensitivity in collies associated with a deletion mutation of the *mdr1* gene. *Pharmacogenetics* 11: 727–33
17. Moore D J 1979 Therapeutic implications of *Babesia canis* infections in dogs. *Journal of the South African Veterinary Association* 50: 346–352
18. Naude T W, Basson P A, Pienaar J G 1970 Experimental diamidine poisoning due to commonly used babesiocides. *Onderstepoort Journal of Veterinary Research* 37: 173–184
19. Onyeyili P A, 1982. *The absorption, distribution and excretion of diazoaminobenzene diaceturate (diminazene) (Ganasang) in goats.* Tuskegee Institute, Alabama
20. Onyeyili P A, Anika S M 1991 Diminazene aceturate residues in the tissues of healthy, *Trypanosoma congolense* and *Trypanosoma brucei brucei* infected dogs. *British Veterinary Journal* 147: 155–162
21. Onyeyili P A, Anika S M 1989 The influence of *Trypanosoma congolense* infection on the disposition kinetics of diminazene aceturate in the dog. *Veterinary Research Communications* 13: 231–236
22. Oppong E N W 1969 Toxicity of berenil in the dog. *Veterinary Record* 85: 370
23. Osborne C A, Finco D R 1995 *Canine and feline nephrology and urology.* Williams and Wilkins, Philadelphia
24. Shakespeare A S 1995 The incidence of canine babesiosis amongst sick dogs presented to the Onderstepoort Veterinary Academic Hospital. *Journal of the South African Veterinary Association* 66: 247–250
25. Stewart C G 1983 A comparison of the efficacy of isometamidium, amicarbalide, and diminazene against *Babesia canis* in dogs and the effect on subsequent immunity. *Journal South African Veterinary Association* 54: 47–51
26. Webb A A, Muir G D 2000 The blood–brain barrier and its role in inflammation. *Journal of Veterinary Internal Medicine* 14: 399–411
27. Welzl C, Leisewitz A L, Jacobson L S, Vaughan-Scott T, Myburgh E 2001 Systemic inflammatory response syndrome and multiple organ damage/dysfunction in complicated babesiosis. *Journal of Veterinary Pharmacology and Therapeutics* 72: 158–62
28. Yamaoka K, Nakagawa T, Uno T 1978 Statistical moments in pharmacokinetics. *Journal of Pharmacokinetics and Biopharmaceutics* 6: 547–558