

Comparative Pharmacokinetics of Diminazene in Noninfected Boran (*Bos indicus*) Cattle and Boran Cattle Infected with *Trypanosoma congolense*†

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The pharmacokinetics of diminazene in five female Boran (*Bos indicus*) cattle before and then during acute and chronic phases of experimental infections with *Trypanosoma congolense* were investigated. A 7.0% (wt/vol) solution of diminazene aceturate (Berenil) was used in all three phases of the study and administered as a single intramuscular dose of 3.5 mg of diminazene base per kg of body weight. There were no significant differences between the values of pharmacokinetic parameters for the noninfected cattle and the values for cattle with a chronic *T. congolense* infection. However, the maximum concentration of the drug in plasma during the acute phase of infection ($8.25 \pm 1.72 \mu\text{g/ml}$) was significantly ($P < 0.01$) greater than that during chronic infection ($5.04 \pm 0.26 \mu\text{g/ml}$) and that in the noninfected state ($4.76 \pm 0.76 \mu\text{g/ml}$). Similarly, the time to maximum concentration of the drug in plasma when diminazene was administered during the acute phase of infection ($18.00 \pm 6.71 \text{ min}$) was significantly ($P < 0.02$) shorter than that for noninfected cattle ($36.00 \pm 8.22 \text{ min}$) and that during chronic infection ($33.75 \pm 7.50 \text{ min}$). The volume of distribution at steady state during acute infection ($1.01 \pm 0.31 \text{ liter/kg}$) was significantly ($P < 0.01$) smaller than that in the noninfected state ($1.37 \pm 0.17 \text{ liter/kg}$) and that in chronic infection ($1.51 \pm 0.24 \text{ liter/kg}$). Eight hours after the drug had been administered, the concentration-time data profiles for each of the three study phases were very similar. Mean concentrations of diminazene in plasma 48 h after administration of the drug were $0.43 \pm 0.07 \mu\text{g/ml}$ in noninfected cattle, $0.43 \pm 0.11 \mu\text{g/ml}$ during the acute phase of trypanosome infection, and $0.44 \pm 0.09 \mu\text{g/ml}$ during the chronic phase of the infection. Results of the present study indicate that the area under the concentration-time curve for diminazene in trypanosome-infected cattle did not differ significantly from that for noninfected cattle. It, therefore, appears that the total amount of diminazene attained and maintained in the plasma of cattle is not significantly altered during infection with *T. congolense*.

The compound 1,3-bis(4-amidinophenyl)triazene diacetate tetrahydrate (7, 14), marketed as Berenil, Ganaseg, and Veriben, is known to have high therapeutic efficacy against a wide range of members of the families *Trypanosomatidae* and *Babesidae* (10). For treatment of *Trypanosoma congolense* and *Trypanosoma vivax* infections in domestic livestock, the recommended dose is 3.5 mg of diminazene aceturate per kg of body weight (b.w.), administered intramuscularly (i.m.) on one occasion. However, while the single dose is usually curative, relapses are known to occur following treatment (21, 27). These are generally attributed either to innate parasite resistance to diminazene or to reinvasion of the peripheral circulatory system by trypanosome populations sequestered in sites believed to be inaccessible to diminazene, such as the central nervous system (20).

The pharmacokinetics of diminazene have been documented for cattle (2, 23, 24), rabbits (18), goats (5), and sheep (4). However, the effect of a trypanosome infection on the pharmacokinetics of diminazene has been described only for rats (38), rabbits (19), and dogs (35). Since such information would have implications for the therapeutic regime used for cattle, the present study was conducted to determine whether the pharmacokinetics of diminazene in noninfected cattle differ significantly from those in cattle with an acute or chronic infection of *T. congolense*.

MATERIALS AND METHODS

Experimental design. The pharmacokinetics of diminazene were determined for five female Boran (*Bos indicus*) cows before and then during acute and chronic infections with *T. congolense*. In each study phase, a single i.m. dose of 3.5 mg of diminazene base per kg of b.w. was administered as a 7.0%, wt/vol, solution (in normal saline) of diminazene aceturate (Berenil [batch 093D574]; Hoechst AG, Frankfurt, Germany). The interval between the first (no infection) and second (acute infection) study phases was 10 weeks, and that between the second and third (chronic infection) phases was 16 weeks. During the second study phase, cattle were infected with *T. congolense* IL 1180 and treated at the first peak of parasitemia. At this stage of infection, the mean packed erythrocyte volume (PCV) ($28.0\% \pm 3.7\%$) was not significantly different from that before infection ($34.0\% \pm 4.5\%$). During the third experimental phase, cattle were infected with *T. congolense* IL 2642. However, in contrast to the second experimental phase, infections were allowed to progress until the mean PCV had fallen to $16.2\% \pm 2.1\%$ before treatment was administered. Hereafter, the terms "noninfected," "acute," and "chronic" refer to the experiments conducted with noninfected cattle, cattle infected with IL 1180, and cattle infected with IL 2642, respectively.

Experimental animals. The ages and body weights (means and standard deviations [SD] given for both) of the cattle used were 18.0 ± 1.4 months and 128.0 ± 22.9 kg before infection, 20.5 ± 1.4 months and 147.2 ± 26.6 kg during acute infection, and 24.5 ± 1.4 months and 157.6 ± 35.7 kg

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during chronic infection. Prior to the beginning of the experiment, serum samples from the animals were examined by an indirect immunofluorescent antibody test (22) and found to be negative for antibodies to *Trypanosoma*, *Theileria*, *Babesia*, and *Anaplasma* species. Throughout the duration of the study, the animals were kept under fly-proof conditions and fed hay and water ad libitum. They were also fed a concentrate ration (Young stock [300 to 400 g per animal per day]; Belfast Millers, Nairobi, Kenya).

Trypanosomes and infection of cattle. *T. congolense* IL 1180 is a doubly cloned derivative (31) of an isolate collected from a lion in the Serengeti National Park, Tanzania (15). *T. congolense* IL 2642 is a doubly cloned derivative (37) of an isolate collected from a cow in Busoga, Uganda (29). Previous studies have shown that when cattle are infected with a population of *T. congolense* and the infection is eliminated with a trypanocide, variant-specific antibody responses are elicited and confer protection against homologous challenge for 5 months (1). Thus, *T. congolense* IL 1180 and *T. congolense* IL 2642 were used in the study described here, since they are antigenically unrelated (36). In order to infect cattle, the numbers of trypanosomes were expanded in sublethally irradiated (650 rads) mice. During the first peak of parasitemia, mice were exsanguinated by cardiac puncture and the blood was collected into sodium citrate (final concentration, 1.0%, wt/vol) to prevent clotting. The blood was pooled and diluted in phosphate-saline-glucose (pH 7.4), and the number of trypanosomes was determined with a Neubauer hemocytometer. The cattle were then infected by intravenous inoculation of 1.0×10^7 trypanosomes per animal. Starting a week prior to initiation of each infection and until 30 days after each treatment, the PCV was determined daily. During the same period, rectal temperature and level of parasitemia (30) were also monitored on a daily basis.

Collection of blood and quantification of diminazene. During each phase of the study, blood was collected at various time intervals (0, 5, 10, 15, 30, and 45 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 21, 24, 30, 36, and 48 h) following administration of the drug. The methods of blood collection, plasma separation and storage, paired-ion extraction of diminazene from the plasma, and quantification of diminazene in the resultant extract using high-performance liquid chromatography were previously described (2, 3). The limit

of detection of the technique is 10 ng of diminazene per ml. Over the concentration range consisting of 0.010, 0.05, 0.10, 0.25, 0.50, 1.00, 2.50, 5.00, and 10.00 $\mu\text{g/ml}$, the response of diminazene was linear ($r^2 = 0.997$). Mean coefficients of variation within and between days were 4.94 and 4.64%, respectively.

Data analysis. The maximum concentration of diminazene in plasma (C_{max}), as well as the time (T_{max}) taken to reach C_{max} , was determined directly from the concentration-time data of samples collected from 0 to 48 h after administration of the drug. Each concentration-time data set was analyzed by least-squares nonlinear regression analysis using computer programs PCNONLIN (40) and STATIS (11). The model of best fit for each data set was selected on the basis of the Akaike (45) and Schwartz (39) information criteria. Initial values for the regression analysis were obtained with an exponential-curve-stripping program, JANA (41). The data were not weighted. The concentration-time data were also analyzed by a noncompartmental approach (17) to calculate the area under the concentration-time curve (AUC) and the area under the first moment of the curve (AUMC); both parameters were determined from zero time to 48 h and were not extrapolated to infinity. AUC, AUMC, mean residence time, apparent total body clearance, and apparent volume of distribution at steady state (V_{ss}) were calculated in accordance with standard equations (17). Bioavailability was calculated and expressed as a percentage of the ratio of AUC from 0 to 48 h determined in the present study (i.m. administration) to AUC from 0 to 48 h for cattle which were treated intravenously with 3.5 mg of diminazene base per kg of b.w. in a previous study (2).

Repeated-measures analysis of variance was used to determine the statistical significance of differences between the means of the pharmacokinetic parameters estimated for the three phases of the study; P values less than or equal to 0.05 were considered significant. Statistically significant differences based on the analysis of variance were further evaluated by the Tukey-Kramer method to make pairwise comparisons among means. All statistical analyses were conducted by using the statistical software package Minitab (28). Half-life ($t_{1/2}$) values were expressed as geometric means (with ranges); values of other parameters were expressed as arithmetic means \pm SD.

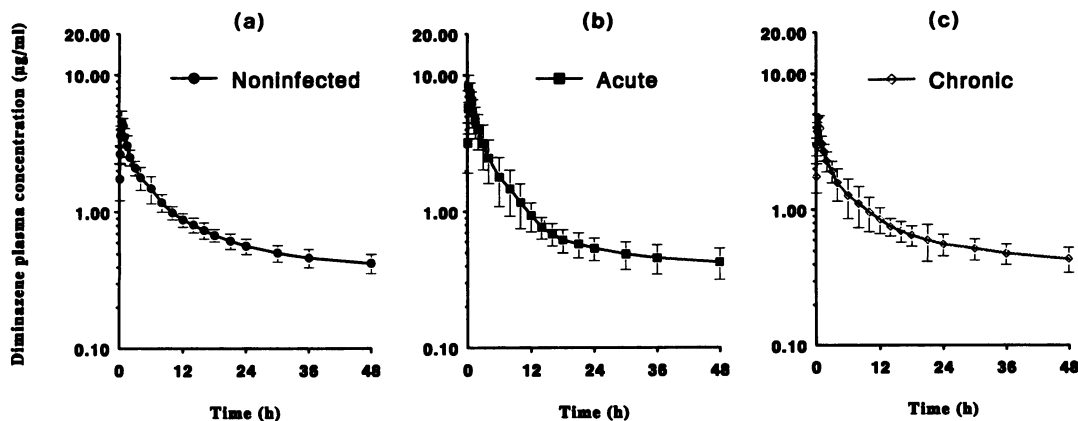


FIG. 1. Absorption and disposition of diminazene in five Boran (*B. indicus*) cows following the administration of a single i.m. dose (3.5 mg/kg of b.w.) before infection (a) and (in the same animals) during acute infection with *T. congolense* IL 1180 (b) and chronic infection with *T. congolense* IL 2642 (c).

TABLE 1. Values of noncompartmental pharmacokinetic parameters following a single i.m. administration of 3.5 mg of diminazene per kg of b.w. to five Boran cows before and after infection with *T. congolense*^a

Infection	C_{max} ($\mu\text{g/ml}$)	T_{max} (min)	AUC ($\mu\text{g} \cdot \text{h/ml}$)	AUMC ($\mu\text{g} \cdot \text{h}^2/\text{ml}$)	MRT (h)	CL ($\text{ml} \cdot \text{min/kg}$)	V_{ss} (liters/kg)	F (%)
None	4.76 \pm 0.76*	36.00 \pm 8.22*	40.81 \pm 4.34	648.34 \pm 79.7	15.89 \pm 1.02	1.44 \pm 0.15	1.37 \pm 0.17*	123.50 \pm 37.47
Acute	8.25 \pm 1.72*‡	18.00 \pm 6.71*‡	49.05 \pm 9.43	658.93 \pm 130.3	13.56 \pm 2.56	1.22 \pm 0.22	1.01 \pm 0.31*‡	141.86 \pm 12.74
Chronic	5.04 \pm 0.26‡	33.75 \pm 7.50‡	38.89 \pm 7.10	639.40 \pm 120.8	16.43 \pm 0.74	1.53 \pm 0.24	1.51 \pm 0.24‡	120.48 \pm 11.09

^a The data are means \pm SD. Values marked with the same symbol (* or ‡) are significantly different ($P < 0.05$). MRT, mean residence time; CL, total body clearance; F, Bioavailability.

RESULTS

Plots of the mean values of the concentration-time data for diminazene in plasma for each of the three phases of the study are shown in Fig. 1. The C_{max} attained when diminazene acetate was administered to animals during the acute phase of infection (*T. congolense* IL 1180) (8.25 \pm 1.72 $\mu\text{g/ml}$) was significantly ($P < 0.01$) greater than that which occurred when the drug was administered during the chronic stage of infection (*T. congolense* IL 2642) (5.04 \pm 0.26 $\mu\text{g/ml}$) and that before infection (4.76 \pm 0.76 $\mu\text{g/ml}$) (Table 1). Similarly, the T_{max} during the acute phase of infection (18.00 \pm 6.71 min) was significantly ($P < 0.02$) shorter than that in noninfected cattle (36.00 \pm 8.22 min) and that during chronic infection (33.75 \pm 7.50 min) (Table 1). Thus, C_{max} and T_{max} did not significantly differ between noninfected cattle and those with a chronic *T. congolense* infection. There were also no significant differences between noninfected cattle and those chronically infected with *T. congolense* in the values of all absorption, distribution, and elimination pharmacokinetic parameters that were determined (Tables 1 to 3). Furthermore, 8 h after the drug had been administered, the mean concentration-time data profiles for each of the three study phases were very similar (Fig. 1). Mean concentrations of diminazene in plasma 48 h after administration of the drug were 0.43 \pm 0.07 $\mu\text{g/ml}$ in noninfected cattle, 0.43 \pm 0.11 $\mu\text{g/ml}$ in the acute phase of trypanosome infection, and 0.44 \pm 0.09 $\mu\text{g/ml}$ in the chronic phase of infection.

Values of the pharmacokinetic parameters estimated through compartmental and noncompartmental analyses are summarized in Tables 1 to 3. Of these parameters, V_{SS} during acute infection (1.01 \pm 0.31 liter/kg) was significantly ($P < 0.01$) smaller than that in the noninfected state (1.37 \pm 0.17 liter/kg) and that during chronic infection (1.51 \pm 0.24 liter/kg). The volume of the central compartment did not differ significantly between the noninfected and trypanosome-infected states. The zero time intercept of the second distribution phase during acute infection (4.97 \pm 2.28 $\mu\text{g/ml}$) was significantly ($P < 0.05$) greater than that in the chronic state (2.06 \pm 0.29 $\mu\text{g/ml}$) and that in the noninfected state

(2.26 \pm 0.50 $\mu\text{g/ml}$). In addition, the transfer rate from the shallow peripheral compartment to the central compartment in noninfected cattle (0.39 \pm 0.10 h^{-1}) was significantly ($P < 0.05$) slower than that during acute infection (0.77 \pm 0.29 h^{-1}). Finally, there were no significant differences between the three phases with respect to AUC, AUMC, mean residence time, or total body clearance (Table 1).

The temperature at the time of treatment during acute infection (40.2 \pm 0.4°C) was significantly ($P < 0.05$) greater than that in the noninfected cattle (38.6 \pm 0.2°C) and that in cattle at the time of treatment during chronic infection (38.2 \pm 0.5°C). Following treatment of acute and chronic infections, all cattle became aparasitemic within 24 h of drug administration and remained aparasitemic throughout the 30-day observation period following treatment.

DISCUSSION

In the work described here, noninfected Boran (*B. indicus*) cattle were administered diminazene i.m. as the commercial preparation Berenil. Kinetic parameters obtained in this study included plasma C_{max} (4.76 \pm 0.76 μg of diminazene per ml), T_{max} (36.00 \pm 8.22 min), absorption $t_{1/2}$ ($t_{1/2k_a}$) (12.22 min), and terminal elimination $t_{1/2}$ ($t_{1/2\lambda_z}$) (86.50 h). These values are similar to the C_{max} (3.2 to 5.9 μg of diminazene per ml), T_{max} (15 to 30 min), and $t_{1/2\lambda_z}$ (40 to 195 h) that were observed for noninfected Holstein-Friesian (*Bos taurus*) cattle that were also administered diminazene via the i.m. route (as Berenil) (23, 24). Despite their similarity to those in previous studies, however, values of parameters determined in the present study were associated with rather wide confidence intervals. But since diminazene has a long elimination $t_{1/2}$ (23, 24), it would appear that some of these parameters may be more precisely estimated by analysis of concentration-time datum points beyond the 48 h used in the present study.

In a previous study (2), it was reported that i.m. administration of diminazene as the diacetate salt to noninfected cattle (same breed and sex as those used in the present study) was followed by rapid absorption of the drug and showed a similar plasma C_{max} (4.68 \pm 1.12 μg of diminazene

TABLE 2. Pharmacokinetic parameters describing absorption and disposition of diminazene following a single i.m. administration of 3.5 mg of diminazene per kg of b.w. to five Boran cows before and after infection with *T. congolense*^a

Infection	Zero time intercept ($\mu\text{g/ml}$)			k_a (h^{-1})	λ_1 (h^{-1})	λ_2 (h^{-1})	λ_z (h^{-1})
	C_1	C_2	C_z				
None	18.58 \pm 4.06	2.26 \pm 0.50*	0.68 \pm 0.14	3.56 \pm 1.33	1.85 \pm 0.66	0.15 \pm 0.09	0.0099 \pm 0.006
Acute	20.02 \pm 0.16	4.97 \pm 2.28*‡	0.58 \pm 0.17	4.51 \pm 0.86	2.70 \pm 0.56	0.24 \pm 0.08	0.0066 \pm 0.005
Chronic	19.97 \pm 0.05	2.06 \pm 0.29‡	0.64 \pm 0.10	3.31 \pm 0.66	2.33 \pm 0.44	0.20 \pm 0.08	0.0083 \pm 0.006

^a The data are means \pm SD. Values marked with the same symbol (* or ‡) are significantly different ($P < 0.05$). λ_1 and λ_2 , rapid and slow exponents, respectively, of the distribution phases. C_1 and C_2 , zero time intercepts of the rapid and slow distribution phases, respectively. C_z , zero time intercept of the terminal elimination phase.

per ml), prolonged $t_{1/2\lambda_z}$ (145.48 h), and significantly different T_{max} (14.00 ± 2.20 min) and $t_{1/2k_e}$ (36.00 min). Similar differences in the pharmacokinetics of the two preparations of diminazene were also reported in studies with goats (5) and sheep (4). The factors responsible for these differences are unknown but may be due to altered elimination of diminazene as a result of inclusion of antipyrine, a chemical stabilizer which constitutes 55.5% of the commercial preparation Berenil (13). However, although it is established that cattle oxidatively metabolize antipyrine into several metabolites which are excreted via urine (44), metabolites of diminazene in animals remain unknown. To date, only unchanged diminazene has been recovered from the liver, feces, urine, and milk of cattle that have been treated with diminazene as the diacetate salt or as Berenil (2, 23, 24).

In the present study, C_{max} and T_{max} in the acute stage of a *T. congolense* infection differed significantly from corresponding values for noninfected cattle and cattle chronically infected with *T. congolense*. The pathophysiological and circulatory changes that accompany trypanosome infections (8, 25) may account for the significantly ($P < 0.01$) higher C_{max} and earlier T_{max} obtained when diminazene was administered to cattle during the acute stage of a trypanosome infection. Furthermore, the possible effects of PCV and parasitemia on the observed differences in C_{max} could not be excluded, since the analytical technique used for quantifying diminazene quantifies intact diminazene only in the plasma. However, since the PCVs in the noninfected and acute-infection groups were not significantly different at the time of treatment, this would indicate that the difference in C_{max} between these two groups was not due to differences in PCV. Finally, the higher C_{max} in acute infection would appear to result from the significantly lower V_{SS} observed for this state compared with those for the noninfected and chronic-infection states (Table 1).

Earlier studies have shown that in noninfected cattle diminazene penetrates erythrocytes, binds to plasma proteins (2, 4, 6), and accumulates in the liver (19, 23). The compound is then eliminated from the body via urine and feces (2, 19, 23). It is not known whether the biochemical and hematological changes which occur when cattle are infected with trypanosomes (32, 42, 43) affect the normal distribution and redistribution of diminazene between the blood and tissues. In the animals used in this study, the V_{SS} in noninfected cattle (1.37 ± 0.17 liter/kg) and that during chronic infection (1.51 ± 0.24 liter/kg) were significantly ($P < 0.05$) greater than that during acute infection (1.01 ± 0.31 liter/kg). Changes in V_{SS} may be associated with changes in drug binding (16). For instance, decreased plasma protein binding of diphenylhydantoin in human uremic patients was associated with significant changes in the drug's apparent V (34). It is, therefore, possible that differences in diminazene's V_{SS} values for different physiological states are associated with a similar phenomenon. Whether this is the case was not investigated in the present study.

Since the trypanocidal activity of diminazene is dependent on uptake of the molecule by trypanosomes (9, 26, 33), it is possible that the presence of a trypanosome infection at the time of treatment may deplete the drug in plasma (12), thereby resulting in an apparent reduction in the elimination $t_{1/2}$ of the drug. However, results from the present study indicate that the AUC for diminazene did not differ significantly between noninfected and trypanosome-infected cattle. Similarly, in studies with rabbits (19) and dogs (35), the concentration-time data for diminazene in plasma did not differ significantly between noninfected animals and animals

TABLE 3. Values of the three-compartment model parameters following a single i.m. administration of 3.5 mg of diminazene per kg of b.w. to five Boran cows before infection and at different stages of infection with *T. congolense*^a

Infection	$t_{1/2}$							V_c (liters/kg)		
	k_e (min)	λ_1 (h)	λ_2 (h)	λ_3 (h)	k_{e1} (h ⁻¹)	k_{12} (h ⁻¹)	k_{13} (h ⁻¹)			
None	12.22 (7.15-16.24)	0.39 (0.25-0.69)	3.94 (2.65-4.77)	86.50 (47.3-354.1)	0.22 ± 0.11	0.85 ± 0.37	0.55 ± 0.23	0.03 ± 0.01	0.39 ± 0.10*	0.17 ± 0.042
Acute	9.35 (7.52-12.87)	0.26 (0.20-0.35)	3.05 (2.16-3.97)	141.60 (57.6-408.0)	0.21 ± 0.13	1.34 ± 0.47	0.59 ± 0.21	0.03 ± 0.02	0.77 ± 0.29*	0.14 ± 0.012
Chronic	12.74 (10.29-16.91)	0.30 (0.24-0.39)	3.67 (2.22-6.20)	107.40 (48.2-388.6)	0.22 ± 0.13	1.09 ± 0.07	0.76 ± 0.24	0.04 ± 0.021	0.43 ± 0.11	0.15 ± 0.002

^a The data for $t_{1/2}$ are geometric means, with ranges in parentheses. All other data are means ± SD. Values marked with an asterisk are significantly different. $t_{1/2\lambda_1}$ and $t_{1/2\lambda_2}$, rapid and slow distribution $t_{1/2}$, respectively; k_{e1} , elimination rate constant from central compartment; k_{12} and k_{13} , transfer rate constants from central to shallow and deep peripheral compartments, respectively; k_{31} and k_{21} , transfer rate constants from deep and shallow peripheral compartments, respectively, to the central compartments; V_c , volume of the central compartment.

infected with *T. congolense*. It would, therefore, appear that the concentration of diminazene that is attained and maintained in treated animals is dependent neither on the presence or absence of a trypanosome infection at the time of treatment nor on the stage of infection when the drug is administered.

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