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Effect of Water Deprivation on the Elimination of Diminazene Aceturate from Blood and Edible Tissues of Goats

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Abstract: The tissue distribution and serum kinetics profile of diminazene aceturate was investigated in non-water deprived and water deprived goats. Water deprivation was observed to increase significantly ($P< 0.05$) the elimination half-life ($t_{1/2}\beta$), distribution half-life ($t_{1/2}\alpha$), area under the curve (AUC) and volume of distribution ($V^d\beta$), and decreased the total body clearance (CL), elimination rate constant (β) and distribution rate constant (α). The drug was distributed to various organs and tissues of the body with the highest concentrations occurring in liver and kidney. Lower concentration of the drug in tissues and organs of water deprived goats at 2 days post injection when compared with those of non-water deprived goat may be due to decreased delivery of the drug to the organs and tissues while the higher concentration noticed in the tissues of water deprived animals later (15 days post treatment) could be due to decreased clearance of the drug from the organs and tissues. Drug residues were still detectable in the tissues of the goats 15 days after drug administration.

Key words: Water deprivation, goats, diminazene, elimination, goats

Introduction

Diminazene aceturate (Berenil) is the drug of choice in the treatment of trypanosomosis and babesiosis in animals (Bauer, 1963; Fairclough, 1963; Pandey and Mishra, 1978). The disposition kinetics of diminazene aceturate have been reported in calves (Kellner *et al.*, 1985), cattle (Klatt and Hadju, 1976), rabbits (Gilbert and Newton, 1982), and dogs (Onyeyili, 1989). Despite its limited use in the treatment of trypanosomosis and babesiosis limited kinetic studies have been carried out with the drug especially in goats deprived of water. Water deprivation could result in dehydration. In sahel region with limited rainfall, animals at times stay for a long period without drinking water. This condition can also occur when animals are sick and are off feed and water. It therefore becomes important to determine the elimination of diminazene aceturate from the blood and edible tissues in water-deprived goats since this will simulate the diseased state or long dry period when animals take little or not drinking water.

The objective of the study therefore was to establish the serum kinetics and extent of distribution of diminazene aceturate in edible tissues of sahel goats. Such information is important in establishing the withdrawal periods of the drug in goats in the sahel region to avoid potential human health hazards following the ingestion of diminazene-treated goats.

Materials and Methods

Experimental animals and treatments: Six healthy sahel goats (1.4 to 2 years old) of both sexes, weighing between 17 to 22 kg were used for the serum kinetics study. They were fed concentrates and hay, and water was provided *ad libitum*. The goats were dosed with diminazene aceturate (Hoechst Farbuerke, Frankfurt, Germany) at 3.5 mg kg^{-1} body weight using the left jugular vein and blood samples collected from the contralateral vein. The goats were thereafter kept for a period of four weeks after which they were deprived of water for 36 h. The drug was then administered to the animals at the same dose into the left jugular vein and blood samples obtained from the right jugular vein.

Twenty-six goats weighing 18 to 23 kg were used for the tissue study. Twenty-four of the animals were separated into two equal groups. One group (non-water deprived) was injected intravenously (I.V.) with the drug at 3.5 mg kg^{-1} while the other group was deprived of water for 36 hours and therefore treated with diminazene at the same dose rate I.V., after which the animals were allowed limited water intake (600 ml per goat per day). The non-treated goats were used for the preparation of control tissues and tissue standards.

Sample collection and drug analysis: Three milliliter of blood was collected from the right jugular vein of each of the six goats at 0.0 (15 min prior to drug administration), 0.08, 0.16, 0.25, 0.5, 0.75, 1,2, 3. 6, 12, 24, 36, 48 and 72 hours after diminazene injection. Blood samples were centrifuged to obtain the serum. Three goats from each group were sacrificed at days 2, 5, 10 and 15 post drug administration and tissues samples (liver, kidney, heart, skeletal muscle and brain) collected. The experimental work area and all utensils were cleaned thoroughly after each slaughter to prevent contamination. All serum and tissue samples collected were frozen until analyzed.

Serum and tissue samples were analyzed for total diminazene according to the calorimetric method of Klatt and Hadjau (1976); the sensitivity of the method was 0.1 μg/g in our laboratory. The colour development portion of the procedure was performed using suitable reagents (Bratton and Marshall, 1939).

Pharmacokinetic analysis: The pharmacokinetic variables – zero time intercept (A and B), concentration at zero time (Cp^o), area under the curve (AUC), rate constants of distribution (α) and elimination (β), total body clearance (CL), volume of distribution ($V^d\beta$), and half lives of elimination ($t_{1/2}\beta$) and distribution ($t_{1/2}\alpha$) were calculated according to standard procedures (Baggot, 1977).

Statistical analysis: Regression analysis, and mean± SD were calculated by means of preprogrammed calculator. Tests for significance between mean parameters in respect of non-water deprived and water deprived goats were performed using the Student's "t" test and the "null" hypothesis was rejected at 5 percent level of probability.

Results

The treatment of goats (both non-water deprived and water-deprived with diminazene intravenously (3.5 mg kg^{-1}) resulted in measurable blood levels for 48 hours (Fig. 1 and 2). Mean peak serum concentrations of 8.1± 0.8 (Fig. 1) and 9.2± 0.7 μg/ml (Fig. 2) of the drug were obtained at 0.08 hr. post drug administration respectively in non-water deprived goats and those deprived of water. The disposition kinetics of diminazene (3.5 mg kg^{-1}) in serum of non-water deprived and water deprived goats are shown in Table 1. The pharmacokinetic evaluation of the drug indicates that the data should fit a two-compartment open model (Fig. 1 and 2). The elimination half-life of 6.30± 1.00 hr. obtained in non-water deprived goats was significantly different from that obtained from goats deprived of water (8.35± 1.08 hours). Goats that were not deprived of water were also observed in this study to have significantly ($P< 0.05$) increased total body clearance (CL), rate constant of elimination (β) and rate constant of distribution (α) and decreased area under the curve (AUC), distribution

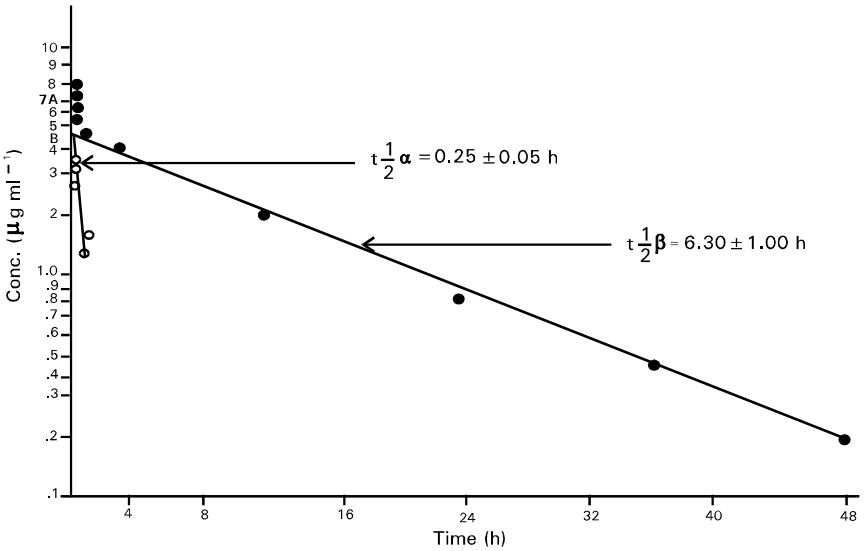

Fig. 1: Serum concentration-time curve for diminazene aceturate (3.5 mg kg^{-1}) following a single intravenous injection to non-water deprived goat

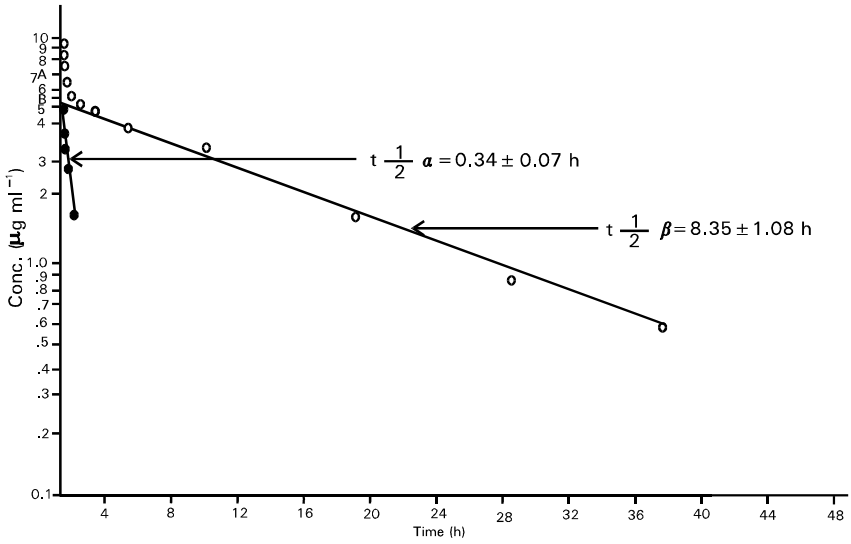

Fig. 2: Serum concentration-time curve for diminazene aceturate (3.5 mg kg^{-1}) following a single intravenous injection to water deprived goats

half life (t½β) and volume of distribution (V_dβ) compared with goats deprived of water (Table 1).

The highest concentrations of 58.28± 4.21 and 48.54± 5.22 µg/g were obtained in the liver of non-water deprived and water deprived goats respectively 2 days after drug administration (Table 2). The concentrations in the liver decreased continuously and at 15 days post administration dropped to 5.51± 0.82 and 28.28± 5.84 µg/g respectively in non-water deprived goats and those deprived of water. The highest concentrations of 31.35± 3.73 and 27.04± 2.11 µg/g (Table 2) were obtained respectively in the kidney of non-water deprived and water deprived goats. Following continuous decrease the concentrations at 15 days after injection of the drug were 1.75± 0.08 and 2.93± 0.44 µg/g in non-water deprived and water deprived goats, respectively. The mean concentrations in muscular tissues (heart and skeletal muscle) were markedly lower than those of the liver and kidney in both non-water deprived and water deprived goats (Table 3).

The skeletal muscle at 2 days had concentrations of 1.34± 0.32 and 1.12± 0.46 µg/g in non-water deprived and water deprived goats respectively and at 15 days post drug administration the concentration was below detectable level in the non-water deprived goats while in goats deprived of water the concentration was 0.12± 0.04 µg/g. Diminazene was only detected in the brain of non-water deprived goats (Table 3). The highest concentration detected was 1.0± 0.07 at 2 days post drug injection, and at day 15, the drug was below detectable level in the brain.

Discussion

The kinetic behaviours of diminazene aceturate in non-water deprived and water deprived goats is best described by two-compartment open model. This agrees with the findings in calves (Kellner *et al.*, 1985), cattle (Klatt and Hadju, 1976), rabbits (Gilbert and Newton, 1982), and dogs (Onyeyili, 1989). The apparent

Table 1: Pharmacokinetic parameters for diminazene following intravenous doses of the drug at 3.5 mg kg^{-1} body weight to non-water deprived and water deprived goats

Parameters	Non-water deprived	Water deprived
A (µg/ml)	7.0± 1.21	7.4± 1.03
B (µg/ml)	4.6± 0.8	5.5± 0.9
Cp° (µg/ml)	11.6± 1.2	12.9± 1.0
AUC (h.µg./ml)	44.3± 5.8	69.2± 9.5
CL (l kg^{-1}/h)	0.92± 0.06	0.63± 0.03
$V^{d}\beta$ (l kg^{-1})	0.27± 0.03	0.75± 0.04
T½ β (h)	6.30± 1.00	8.35± 1.08
(T½ α (h)	0.25± 0.05	0.34± 0.07
β (hr^{-1})	0.110± 0.02	0.083± 0.008
α (hr^{-1})	2.80± 0.6	2.03± 0.6

A = Zero time intercept for distribution
B = Zero time intercept for elimination
Cp° = Zero time serum concentration
AUC = Area under the serum concentration curve
Cl = Total body clearance
$V^{d}\beta$ = Volume of distribution
$t_{½}\beta$ = Elimination half-life
β = Elimination rate constant
$t_{½}\alpha$ = Distribution half-life
α = Distribution rate constant

volume of distribution ($V^{d}\beta$) relates the drug level in the serum to the total amount of drug in the body after the attainment of distribution equilibrium. The volume of distribution in non-water deprived goats (0.27± 0.03 1 kg^{-1}) was significantly lower than that of goats deprived of water (0.75± 0.04 1 kg^{-1}). This is an indication of more extensive distribution of the drug in goats deprived water compared to those of non-water deprived goats. The more extensive distribution in goats deprived of water may be suggestive of slower elimination of the drug in water deprived goats. It is a fact that the greater the volume of distribution, the longer the half-life of elimination ($t_{½}\beta$) and the slower will the drug be eliminated from the body. Water deprivation is known to decrease the total body water, blood and plasma volumes and extracellular and intracellular fluid volumes (Macfarlane *et al.*, 1961; Little *et al.*, 1976; Ummuna *et al.*, 1981; Hassan, 1989; Meinfjes and Engelbrecht, 1994). Total serum or plasma proteins and albumin concentrations are also known to increase with water deprivation (Anganga *et al.*, 1989; Hassan, 1989; Igbokwe and Ajuzieogu, 1991). The increased serum albumin as a result of water deprivation could enhance drug protein binding, and this could be a factor in the differences in volume of distribution of diminazene noticed in water deprived goats in the present study as compared to that of non-water deprived animals. The more extensive volume of distribution following water deprivation may explain the higher elimination half-life ($t_{½}\beta$) and lower values of elimination rate constant (β) and total body clearance (CL) obtained in goats deprived of water ($t_{½}\beta$ = 8.35± 1.08 hr; β = 0.083 ± 0.008 h^{-1}, CL = 0.63± 0.03 l kg^{-1}/hr) compared to non-water deprived value of goats ($t_{½}\beta$ = 6.30± 1.0 hr; β = 0.110± 0.02 h^{-1}; CL = 0.92± 0.06 l kg^{-1}/hr). The lower value of total body clearance in water deprived goats may also have resulted from decreased blood flow to the kidney and other organs of elimination due to inadequate blood volume (Igbokwe, 1993). Decreased blood volume as a result of water deprivation may also explain the lower value of distribution rate constant (α) and higher value of distribution half-life ($t_{½}\alpha$) in goats deprived of water (α = 2.03± 0.6 h^{-1}; $t_{½}\alpha$ = 0.34± 0.07 h) compared to the animals that were not deprived of water (α = 2.8± 0.6 h^{-1}; $t_{½}\alpha$ = 0.25 ± 0.05 hr). However, despite the differences noticed with respect to the other kinetic parameters between the water deprived and non-water deprived goats, it is worthy of note that the concentration of the drug at zero time (Cpo) which is the sum of the zero time intercepts for distribution (A) and elimination (B), in non-water deprived goats did not differ significantly (P> 0.05) from those of water deprived goats.

The results presented in this study also show that diminazene aceturate is readily distributed to various organs and tissue of goats. Highest concentrations of the drug occurred in the excretory organs (liver and kidneys). This is in agreement with the observations in dogs (Onyeyili, 1989) and cattle (Klatt and Hadju, 1976). The high concentration obtained in the liver and kidneys are not unexpected, the liver being the principal organ of bio-transformation while the kidney is the main organ of elimination. The contractions in the muscular tissues were lower than those of the excretory organs in both the non-water deprived and water deprived goats in this study. These lower concentrations

Table 2: Mean* diminazene concentrations (µg/g) in the liver and kidney of non-water deprived and water deprived goats treated intravenously with the drug at the rate of 3.5 mg kg^{-1}

	Concentrations (µg/g)			
	Liver		Kidney	
Time (days)	Non-water deprived	Water deprived	Non-water deprived	Waterdeprived
0	0.00	0.00	0.00	0.00
2	58.28± 4.21	48.54± 5.22	31.35± 3.73	27.04± 2.11
5	34.68± 4.73	44.28± 4.03	27.43± 2.53	25.88± 1.76
10	24.42± 2.50	36.26± 4.12	14.30± 2.23	5.02± 0.84
15	5.51± 0.82	28.28± 5.84	1.75± 0.08	2.93± 0.44

Table 3: Mean* diminazene concentrations (µg/g) in the heart, skeletal muscle and brain of non-water deprived and water deprived goats treated intravenously with the drug at the rate of 3.5 mg kg^{-1}.

	Concentrations (µg/g)					
	Heart		Skeletal		Brain	
Time (days)	Non-water deprived	Water deprived	Non-water deprived	Water deprived	Non-water deprived	Waterdeprived
0	0.00	0.00	0.00	0.00	0.00	0.00
2	0.42± 0.20	0.37± 0.11	1.34± 0.32	1.12± 0.46	1.0± 0.07	0.00
5	0.27± 0.06	0.55± 0.11	0.45± 0.08	0.45± 0.06	0.50± 0.03	0.00
10	0.14± 0.05	0.34± 0.04	0.22± 0.06	0.36± 0.10	0.2± 0.10	0.00
15	0.14± 0.03	0.32± 0.06	0.00± 0.00	0.12± 0.04	0.00	0.00

*Mean ± S.D. based on three observations.

observed in the muscular tissues appear to agree with the observations in cattle and dogs (Klatt and Hadju, 1976; Onyeyili, 1989). Substantial concentrations of diminazene was observed to be present in some edible tissues at 15 days post drug administration, with the concentrations in water deprived goats being higher than those of non-water deprived goats at this time period. The initial lower concentrations noticed in water deprived goats when compared with values in non-water deprived goats may be due to decreased blood flow to the various organs and tissues because of water deprivation which may have reduced the blood volume. The high tissue and organ concentrations of the drug in water deprived goats later may be due to decreased clearance of the drug from the organs and tissues as a result of impaired circulatory function due to deceased water intake. Diminazene was observed to persist in some edible tissue of both water deprived and non-water deprived goats for more than 15 days after intravenous injection. This should be given due consideration in estimation of the withdrawal period for the drug in this specie. In conclusion, water deprivation as in this study influenced the various kinetic parameters and also affected the extent of delivery and clearance of the drug from organs and tissues of the body.

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References

Anganga, A.A., N. N. Umunna, E. O. Onyedipe and P. N. Okoh, 1989. Influence of intermittent watering on some blood components of grazing Yankasa sheep. Nig. J. Anim. Prod., 15: 77-82.

Baggot, J.D., 1977. Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary Clinical Pharmacology. W. B. Saunders Company, Philadelphia, pp: 45-327.

Bauer, F., 1963. Veterinary Preparations (Hoechst). Blue Book for the Veterinary Profession, 7: 26-30.

Bratton, A.C. and E.K. Marshall, 1939. A new coupling component for sulfanilamide determination. J. Biol. Chem., 128: 537-550.

Fairclough, R., 1963. A comparison of metamidium, samorin, berenil and ethidium bromide under field condition in Kenya. Vet. Rec., 75: 855-858.

Gilbert, R. J. and B. A. Newton, 1982. Pharmacokinetics and efficacy of the trypanocide diminazene aceturate (Berenil) in rabbits. Vet. Rec., 111: 397.

Hassan, G.E.A., 1989. Physiological responses of Anglo-Wubian and Balami goats and their cross breeds to water deprivation under subtropical condition. Liv. Prod. Sci., 22: 295-305.

Igbokwe, I.O., 1993. Haemoconcentration in Yankasa sheep exposed for prolonged water deprivation. Small Rum. Res., 12: 99-105.

Igbokwe, I.O. and G.I. Ajuzieogu, 1991. The effect of prolonged water deprivation on some clinical and haematological parameters of Yankasa sheep in the arid sahel region of Nigeria. J. Arid Agric., 3: 134-139.

Kellner, H.M., H.G. Eckert and M.H. Volz, 1985. Studies in cattle on the disposition of the antitrypanosomal drug diminazene diaceturate (Berenil). Trop. Med. Parasit, 36: 199-204.

Klatt, P. and P. Hadju, 1976. Pharmacokinetic investigations on diminazene and roliteracycline in comparison to a combination of both. Vet. Rec., 99: 372-374.

Little, W., B.F., Samson, R. Manston and W.M. Allen, 1976. The effects of restricting the water intake of dairy cows upon their milk yield, body weight and blood composition. Anim. Prod., 22: 329-339.

Macfarlane, W.V., R.J.H. Morris, B. Howard, J. McDonald and O.G. Budzolsen, 1961. Electrolyte changes in tropical Merino sheep exposed to dehydration during summer. Aus. J. Agric. Res., 12: 889-912.

Meinfjes, R.A. and H. Engelbrecht, 1994. The effect of short term dehydration on kidney function, plasma protein concentration, faecal water loss and total body water in sheep. S. Afr. J. Sci., 90: 27-32.

Onyeyili, P.A., 1989. Comparative chemotherphy and pharmacokinetics of canine trypanosomiasis. Ph.D. Thesis, University of Nsukka, Nigeria.

Pandey, N.N. and S.S. Mishra, 1978. Comparative efficacy of berenil, acriflavin and sulfadimethoxine in *Babesia bigemina* infection in indigenous cow calves. India Vet. J., 55: 144-148.

Ummuna, N.N., C.N. Chineme, D.I. Soror, A. Ahmed and S. Abed, 1981. Response of Yankasa sheep to various lengths of water deprivation. J. Agric. Sci., 96: 619-622.