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Pharmacokinetics of Diminazene Aceturate in Buffalo Calves

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Abstract

The pharmacokinetic study of diminazene aceturate (DMZ) was carried out in two separate groups of 4 each clinically healthy female Murrah buffalo calves after single dose i.v. (8 mg/kg) and i.m. (16 mg/kg) administration. The mean free peak serum concentration of DMZ (CS_{max}) after i.v. (26.28±0.67µg/ml) and i.m. (8.41±2.43 µg/ml) administrations were obtained at t_{max} of 5 and 30 min respectively. The DMZ serum concentrations time data were best fitted to the two compartment open model. The calculated serum half life ($t_{½}$ β) values of DMZ were 15.099±2.504 and 14.225±2.682 h after i.v. and i.m. administration respectively. The mean values of total body clearance rate of DMZ (ClB) after i.m. (3.785±1.119 ml/kg/min) was significantly higher ($P<0.05$) as compared with the i.v. (0.537±0.063 ml/kg/min). DMZ was highly bound (77.14 to 94.40%) to buffalo calf plasma protein and its penetration into erythrocytes increased with increasing concentrations in blood (7.60 to 33.00µg/ml). Based on pharmacokinetic profiles, the satisfactory dosage regimens of diminazene aceturate in buffalo calves were derived (2 mg/kg, i.v. and 12mg/kg, i.m). In case of emergent disease conditions to ensure high DMZ serum concentrations, i.v. route may be preferred over i.m. route.

Keywords: Diminazene aceturate; Buffalo calves; Pharmacokinetics

Abbreviations: A&B: Zero time drug concentration in serum during distribution / absorption and elimination phases respectiely; C°s: Theoretical drug concentration in serum at zeero time; $t_{½}$: Half life of drug in distribution, absorption and elimination phases respectively; AUC: Total area under curve; K_{12}, K_{21} & K_2: Micro rate concentration for drug transfer from central to peripheral, peripheral to central and elimination frm the central compartment respectively; FC: Fraction of drug available for elimination from the central compartment; T/P: Tissue to Plasma concentration ration of the drug; Vd_c, Vd_B and Vd_{area}: The apparent volume of dristribution, based on elimination and based on total area under curve respectively of the drug; Cl_B: Total body clearance rate of the drug

Introduction

Diminazene aceturate (DMZ), an aromatic diamidine is the drug of choice for babesiosis in livestock. Babesiosis poses a major health problem for cattle, buffalo, sheep and goats in all the tropical and subtropical regions of the world. Its usefulness and tolerance in animals have been documented (Sardar et al., 1994).

The pharmacokinetic studies on DMZ have been carried out in sheep (Aliu and Odegaard, 1985), goats (Aliu et al.,1984), bovine calves (Sardar et al., 1995), cow (Kellner et al.,1985), rats (Raether et al.,1972) and rabbits (Gilbert, 1983). The information on the disposition kinetics of DMZ in buffalo although important to be known is not available. This was possibly due to the fact that buffalo being an unique species of Indian subcontinent did not attract much attention of workers abroad. It may be mentioned here that in Chotanagpur plateau as well as in other hilly tracts of India, the buffalos are the principal milch and draft animals. In view of the above, the present work was designed to explore and identify the disposition kinetics of DMZ in buffalo calves in order to facilitate its proper clinical usages.

Materials and Methods

Animals

The pharmacokinetic study of diminazene aceturate after i.v. and i.m. administrations was conducted on 2 groups of 4 each clinically healthy 1.5 - 2 years female Murrah buffalo calves (150 -160 kg) of Ranchi Veterinary College dairy farm. The experimental animals were kept at room temperature in the faculty dairy farm were fed on standard rations, partial grazing (Panday and Roy,1998) and has free access to water.

Drugs used

Diminazene aceturate (Dimaze®, Merind Limited, Mumbai, India). DMZ granules 5G was dissolved in 30 ml water for injection and was injected as a single i.v. (8 mg/kg) and i.m. dose (16 mg/kg) in the right jugular vein and gluteal muscles respectively to the experimental buffalo calves of the corresponding animal's group.

Collection of experimental samples

Blood samples (5 ml) were collected from the left jugular vein by veinipuncture in clean test tubes from each experimental buffalo calf at 5, 10, 15, 30 min and 1, 2, 3, 6, 12, 24 and 48 h after single dose i.v. (8 mg/kg) and at 5, 10, 15, 20, 30min and 1, 2, 3, 4, 6, 8, 12, 24 and 48 h after i.m. (16 mg/kg) administra-

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tion. Serum was separated after keeping the tubes in slanted position for 4- 5 h at room temperature. The predrug administration blood samples in sufficient quantity from each experimental buffalo calf were collected and serum separated. These serum samples served as analytical controls and were also used for preparation of DMZ standards.

Predrug administration (0 h) blood sample 3 to 5 ml from each experimental buffalo calf were also collected in heparinized test tubes for separation of plasma for DMZ plasma protein binding study. Plasma from these blood samples were separated by centrifugation at 2,500 G for 10 min.

Analytical methods

The analysis of DMZ in serum samples was carried out by the colorimetric method reported by Raether et al., 1972. The limits of detection was 0.05 µg/ml diminazene aceturate.

Pharmacokinetic calculations

The pharmacokinetic parameters of DMZ in buffalo calves were calculated by the Personal Computor following the method described by (Gibaldi et al., 1969; Notari, 1980; Baggot, 1977).

In-vitro Plasma Protein Binding

The in-vitro plasma protein binding of DMZ in buffalo calves was determined by the equilibrium dialysis technique (Davis, 1943).

In-vitro Erythrocyte up-take

The in-vitro erythrocyte up-take of DMZ in buffalo calves blood was studied by the method of Michel (1949) modified by Oser (1965).

Statistical Analysis

Data are presented as mean ±S.E. Significance of the observed differences in the pharmacokinetic parameters between i.v. and i.m. groups was assessed by students unpaired t-test (Snedecor and Cochran, 1964). A value of $P<0.05$ was considered statistically significant.

Results

The mean comparative total and free serum concentrations of DMZ and percent metabolite in buffalo calves after single dose i.v. and i.m. administrations are presented in Table 1. The mean

Time	Intravenous			Intramucular		
	Total	Free	% Metabolite	Total	Free	% Metabolite
5 min	28.15±0.47	26.28±0.67	6.67± 1.61	1.53±0.10	1.33±0.07	2.63±2.89
10min	21.80±1.16	19.02±0.29	11.49± 2.51	4.44±0.07	1.78±0.03	59.88±0.94
15min	18.59±0.99	15.25±0.96	15.98± 4.78	4.85±0.06	2.15±0.10	82.34±29.94
20min	N.A.	N.A.	N.A.	7.54±0.10	3.30±0.08	56.22±1.20
30min	16.25±0.99	13.22±0.69	14.57± 2.06	8.93±2.58	8.41±2.43	5.81±2.50
1hr	14.38±0.69	11.88±0.91	17.47± 4.08	6.28±0.38	5.58±0.25	25.88±11.88
2hr	12.94±0.60	10.70±0.19	16.67± 3.71	3.56±0.28	2.48±0.26	30.65±4.83
3hr	12.21±0.09	10.03±0.60	17.80± 5.19	3.61±0.26	2.60±0.08	25.37±4.32
4hr	N.A.	N.A.	N.A.	3.08±0.02	2.53±0.04	17.87±1.36
6hr	11.31±0.39	9.05±0.31	19.52± 4.25	2.86±0.12	2.19±0.08	23.27±1.02
8hr	N.A.	N.A.	N.A.	2.51±0.10	2.15±0.14	14.79±2.35
12hr	7.95±0.78	5.83±0.18	24.60± 5.44	1.33±0.42	0.94±0.28	20.68±7.02
24hr	4.09±0.52	2.64±0.49	36.35± 8.57	1.09±0.01	0.33±0.22	79.06±14.45
48hr	2.89±0.64	1.84±0.26	31.87± 6.12	1.01±0.07	0.27±0.18	75.24±16.96

N.A. - Not analysed

Table1: Comparative mean (n=4) serum concentration (µg/ml) of diminazene in buffalo calves after i.v. (8mg/kg) and i.m. (16mg/kg) administration.

Parameters	Intravenous		Intramuscular	
	Total	Free	Total	Free
A (µg/ml)	31.710±6.615	29.546±2.918	2.978±1.058*	2.328±0.357**
B(µg/ml)	13.887±0.948	12.056±0.856	3.812±0.352**	3.169±0.188**
C^os(µg/ml)	45.598±7.072	41.602±3.505	6.790±1.402**	5.288±0.517**
α (h)	9.035±2.595	8.951±1.465	5.370±3.300	4.377±0.526*
$t_{1/2}$ α (h)	0.114±0.047	0.083±0.011	0.636±0.461	0.165±0.020
β (h^{-1})	0.036±0.006	0.050±0.008	0.051±0.019	0.053±0.009
$t_{1/2}$ β (h)	21.191±4.060	15.099±2.504	19.301±5.662	14.225±2.682
AUC (μgml^{-1} .h)	421.195±70.394	258.965±30.380	97.407±23.753*	114.322±54.260
K_{12}(h^{-1})	6.263±1.986	6.201±1.061	2.621±1.891	1.86±0.298*
K_{21}(h^{-1})	2.684±0.671	2.631±0.416	2.703±1.395	2.481±0.252
K_2(h^{-1})	0.124±0.036	0.168±0.026	0.097±0.047	0.078±0.027
Fc	0.324±0.038	0.296±0.017	0.602±0.057	0.572±0.024
T/P	2.237±0.423	2.412±0.212	-	-
Vd_c (LKg^{-1})	0.188±0.028	0.196±0.016	-	-
Vd_B (LKg^{-1})	0.584±0.039	0.673±0.043	-	-
Vd_{area} (LKg^{-1})	0.578±0.038	0.664±0.042	-	-
Cl_B($mlKg^{-1}min^{-1}$)	0.537±0.063	0.405±0.940*	3.785±1.119**	0.342±0.051

*p<0.05 **p<0.01

Table 2: Comparative mean values (n=4) of kinetic parameters of diminazene in buffalo calves after single i.v. (8mg/kg) and i.m. (16mg/kg) administration.

A & B, Zero time drug concentration in serum during distribution / absorption and elimination phases respectiely; C^os ,Theoretical drug concentration in serum at zeero time; $t_{1/2}$ β, Half life of drug in distribution, absorption and elimination phases respectively; AUC, Total area under curve; K_{12}, K_{21} & K_2,Micro rate concentration for drug transfer from central to peripheral, peripheral to central and elimination frm the central compartment respectively; FC, Fraction of drug available for elimination from the central compartment ; T/P, tissue to plasma concentration ration of the drug; Vd_c, Vd_B and Vd_{area},The apparent volume of dristribution, based on elimination and based on total area under curve respectively of the drug; Cl_B, Total body clearance rate of the drug.

comparative pharmacokinetic values of DMZ after single dose i.v. and i.m. administrations are presented in Table 2. The in-vitro plasma protein binding and up-take of diminazene aceturate by buffalo calf erythrocytes have been shown in Table 3 and Table 4 respectively.

Discussion

DMZ serum concentrations

The mean free serum concentrations of diminazene aceturate obtained after single i.v. dose in buffalo calves showed higher value of Cs_{max} as compared to the i.m. administration (Table 1). The higher value of Cs_{max} after i.v. dose was expected because of complete bio-availability of the compound into the systemic circulation (Baggot, 1977). The capillary endothelial tissue forms a barrier against drug entry into systemic circulation after i.m. administration. However, in this experiment, the aquous solution of DMZ (7.5%) was injected i.m. into the gluteal muscle. The rate of absorption of DMZ administered i.m. in buffalo calves in this study would have mainly depended on the vascularity of the injection site. Other factors governing the rate of absorption include the degree of ionization and lipid solubility of the compound, molecular size of the lipid insoluble substances and the area over which the injection is given (Schou, 1961; Sund and Schou, 1964). Absorption of compounds from aquous solutions injected i.m. is relatively rapid and the peak concentration in serum is usually reached within 30 min. to 1 h. The results obtained in this study also evidenced the rapid absorption of diminazene with a peak serum concentration at 30 minute (Table 1).The calculated value obtained for absorption rate constant (K_a) for free DMZ (4.377±0.526 h^{-1}) also strengthened the above statement that DMZ was rapidly absorbed in buffalo calves. Sardar et al. (1994) reported a Cp_{max} of 30.25±1.37µg/ml after single dose i.v. (16mg/kg) administration of DMZ in bovine calves. The Cs_{max} (8.41±2.43 µg/ml) obtained in this experiment after single i.m. dose (16 mg/kg) in buffalo calves was higher than that reported by Mamman and Peregrine, (1994) in goats after single i.m. dose (3.5 mg/kg). The results obtained after single i.m. dose showed that diminazenz was rapidly absorbed and produced required therapeutic concentration at 5 minute (1.33±0.07µg/ml).

Acetylation

The rate of acetylation as observed from the % metabolite at various DMZ serum concentration time data in this study (Table 1) did not increase or decrease in the mathematical order. However, lower DMZ total serum concentrations obtained at later points of time both after i.v. and i.m. administrations in this study could have been due to increased rate of acetylation. Thus the rate constants of acetylation of DMZ in buffalo did not obey consistently the "0" or "1" order kinetics model in this study. Such observation with respect to acetylation of sulphonamides in dairy cattle have been also reported (Stowe and Sisodia, 1963).

Pharmacokinetics

The DMZ serum concentration time data obtained in buffalo calves in this study were best fitted to a 2-compartment open pharmacokinetic model. The C^o_s value (41.60±3.50 µg/ml) of free DMZ obtained after single i.v. dose was approximately 8 times higher as compared with (5.28±0.51 µg/ml) i.m. administration. However, the Cs_{max} (28.15±0.47 µg/ml) obtained after i.v. was approximately 3 times higher as compared to the Cs_{max} (8.41±2.43 µg/ml) after i.m. injection in buffalo calves. It may be mentioned that the C^o_s is a hypothetical parameter being the sum of A and B, can differ with a little variation in selection of points pertaining to elimination phase. The higher value obtained for Ka (4.377±0.526 /h) along with a corresponding lower value of absorption half life ($t_{½}$ Ka) shows that DMZ was rapidly absorbed after i.m. injection in buffalo calves and could maintain therapeutic concentration upto 48 h. The data of $t_{½}$ Ka shows that half life of the amount of DMZ injected by i.m. route was absorbed in 10 minute.

The higher values obtained for α (8.951±1.465 h^{-1}) and Ka (4.377±0.526 h^{-1}) and the subsequent lower value for $t_{½}$ α (0.083±0.011 h) and $t_{½}$ Ka (0.165±0.020 h) denoted a rapid distribution and absorption of DMZ in the body fluids of buffalo calves. Mallick et al. (1998) have reported the values for α and $t_{½}$ α of DMZ in goats as 2.39±0.12 h^{-1} and 0.29±0.25 h respectively. The rapid distribution and absorption of DMZ was further strengthened by the higher sum of K_{12} and K_{21} than the K_2 as depicted in Table 2.

The apparent volume of distribution, based on elimination and total area under curve of DMZ for i.v. and i.m.routes in buffalo calves derived were 0.196±0.016, 0.673±0.043 and 0.664±0.042 and 3.113±0.299, 5.455±0.301 and 5.499±0.287 L Kg^{-1} respectively. The consistant higher values obtained for the apparent volume of distribution showed that this compound perfused well in to the body spaces of buffalo species. The volume of distribution (Vd) of DMZ in goats has been reported to be 1.464±0.053 L Kg^{-1} (Mallick et al., 1998). The derived Cl_B of DMZ after i.v. and i.m. routes were 0.537±0.063 and 3.785±1.119 ml/kg/min respectively. Sardar et al. (1994) have reported the Cl_B value of this compound after i.m. dose in bovine calves as 0.564±0.090 ml/kg/min.

The approximately equal values of $t_{½}$ β obtained after i.v. (15.099±2.50 h) and i.m. (14.225±2.682 h) envisaged first-order kinetics of DMZ in buffalo species. Sardar et al. (1994) reported $t_{½}$ β value equal to 31.604±2.296 h in bovine calves after single i.m. dose (16mg/kg) which was twice the value obtainedin this experiment for buffalo calves. Mallick et al. (1998) reported $t_{½}$ β value of DMZ equal to 13.49±1.26 h in goats after single i.v. dose (8mg/kg) which is closer to the value of $t_{½}$ β obtained in this experiment.The overall kinetic variables of total DMZ for i.v. and i.m. routes derived in buffalo calves in this study were comparatively higher than their respective values for free DMZ. Based on the pharmacokinetic variables obtained in this study, the satisfactory dosage regimen of diminazene aceturate in buffalo was derived (2mg/kg i.v. and i.m.).

Plasma Protein Binding

Diminazene was observed to be highly bound to plasma proteins of buffalo calves (77 to 94.4%) in the concentration range of 6.25 to 100 µg/ml (Table 3).

Erythrocytes' uptake

The uptake of DMZ by buffalo calves erythrocytes has been found to range between 7 to 33 µg/ml in 8.5 × 10^6/ml at the

Plasma concentrations (µg/ml)	Plasma protein bound drug (%)
6.25	77.14
12.50	88.80
25.00	94.05
50.00	94.40
100.00	91.30

Table3: In-vitro plasma protein binding value of diminazene in buffalo calves.

Blood concentration (µg/ml)	After washing total count of (RBCx10^6/ml)	Concentration (µg/ml)	Concentration (µg/ml in 8.15x10^6/ml)
6.25	6.7x10^6	6.25	7.60
12.50	6.4x10^6	11.50	14.64
25.00	5.6x10^6	14.20	20.66
50.00	5.9x10^6	17.32	23.92
100.00	5.4x10^6	21.87	33.00

Table 4: The up-take of diminazene by buffalo calf erythrocytes (Total count of RBC in buffalo calf blood was 8.15x106/ml).

blood concentration range of 6.25 to 100 µg/ml. DMZ showing a sustained rise with concentration with a maximum at 100 µg/ml. The passage of DMZ into erythrocytes and its accumulation there in provides an added benefit from the clinical point of view against intra erythrocytic protozoan parasites susceptible to this compound (Table 4).

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References

1. Aliu YO, Odegaard S, Sognen E (1984) Diminazene Berenil: Bioavailability and disposition in dairy goats. Acta veterinaria Scandinavia 25: 593-596. » CrossRef » PubMed » Google Scholar
2. Aliu YO, Odegaard S (1985) Pharmacokinetics of diminazene in Sheep. J Pharmacokinet Biopharm 13: 173-184. » CrossRef » PubMed » Google Scholar
3. Baggot JD (1977) Principles of drug disposition in domestic animals. The basis of Veterinary Clinical Pharmacology, W B Saunders Co.,Philadelphia, London, P73 -112. » CrossRef » PubMed » Google Scholar
4. Davis BD (1943) The binding of sulphonamide drugs to plasma proteins: A factor in determining the distribution of drugs in the body. J Clin Invest 22: 753-762. » CrossRef » PubMed » Google Scholar
5. Gibaldi M, Nagashima R, Levy G (1969) Relationship between drug concentration in plasma or serum and amount of drug in the body. J Pharm Sci 58:193 -197. » CrossRef » PubMed » Google Scholar
6. Gilbert RJ (1983) Studies in rabbits on the disposition and trypanocidal activity of the anti-trypanocidal drug. Diminazenz aceturate (Berenil). Br J Pharmacol 80: 133-139. » CrossRef » PubMed » Google Scholar
7. Kellner HM, Eckert HG, Volz MH (1985) Studies in cattle on the disposition of the antitrypanosomal drug diminazene. Tropical Medical Parasitology 36: 199-204. » CrossRef » PubMed » Google Scholar
8. Mallick TK, Sardar KK, Parija SC, Patel MB, Mishra SN (1998) Pharmacokinetics of diminazene in Black Bengal goats. International Journal of Animal Science 13: 95 -98. » CrossRef » PubMed » Google Scholar
9. Mamman M, Peregrinc AS (1994) Pharmacokinetics of diminazene in plasma and cerebrospinal fluid of goats. Research in veterinary science 57: 253-255. » CrossRef » PubMed » Google Scholar
10. Michel HO (1949) Journal of laboratory clinical medicine 34: 1564-1565. » CrossRef » PubMed » Google Scholar
11. Notari RE (1980) In Biopharmaceuticals and clinical pharmacokinetics 3rd edn. Marcel Dekker. Inc, New York P16-106. » CrossRef » PubMed » Google Scholar
12. Oser BL (1965) In Hawk's Physiological Chemistry. 14th edn. Tata McGrew Hill Publishing Co. Ltd., New Delhi. » CrossRef » PubMed » Google Scholar
13. Panday SN, Roy BK (1998) Disposition kinetics of mebendazole in plasma, milk and ruminal fluids of goats. Small ruminant research 27: 111-117. » CrossRef » PubMed » Google Scholar
14. Raether W, Hajdu P, Seidenath P, Damm D (1972) Pharmacokinetische and chemotophylaktische untersuchungen mit Berenil an Wister-Rattanrypanosoma rhodesiense, Z tropenmed Parasitolgy 23: 418-427. » CrossRef » PubMed » Google Scholar
15. Sardar KK, Parija SC, Mishra SN (1994) Pharmacokinetics of diminazene in bovine calves. Indian Veterinary Journal 74: 558-560. » CrossRef » PubMed » Google Scholar
16. Sardar KK, Parija SC, Mishra SN (1995) Diminazene Bioavailability and disposition in bovine calves. Indian Journal of Animal Science 65: 857 -859. » CrossRef » PubMed » Google Scholar
17. Schou J (1961) Absorption of drugs from subcutaneous connective tissue. Pharmacological Review 13: 441-464. » CrossRef » PubMed » Google Scholar
18. Snedecor GW, Cochran WG (1964) Statistical Methods. 6th edn. Oxford IBH Publishing, Calcutta, India. » CrossRef » PubMed » Google Scholar
19. Stowe CM, Sissodia CS (1963) The pharmacological properties of sulfadimethoxine in dairy cattle. Am J Vet Res 24: 525-535. » CrossRef » PubMed » Google Scholar
20. Sund RB, Schou J (1964) The determination of absorption rates from rat muscles an experimental approach to kinetic description. Acta Pharmacol Toxicol 21: 313-325. » CrossRef » PubMed » Google Scholar