International Journal of Pharmaceutical Sciences and Drug Analysis

E-ISSN: 2788-9254
P-ISSN: 2788-9246
www.pharmacyjournal.info
IJPSDA 2022; 2(1): 15-19
Received: 13-11-2021
Accepted: 15-12-2021

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Plasma kinetics profile of Nigerian indigenous dogs concurrently treated with diminazene aceturate and oxytetracycline long acting

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Abstract
Concurrent administration of diminazene aceturate (DA) and oxytetracycline-long acting (oxyt-LA) to dogs often results in nervous signs and death of the animals, whereas oxytetracline-short acting is not associated with such negative effects. To verify the cause, one group comprising 6 dogs was treated with DA alone at dose of 3.5 mg/kg body weight, intravenously (IV) while a second group of 6 six dogs, was given DA intravenously, followed by Oxyt-LA, ten minutes later, at dose of 20 mg/kg, intra-muscularly. Blood was collected from each dog in the two groups, post treatment (PT) at: 0.25, 0.5, 1, 2, 3, 6, 9, 12, 24, 36, 48, 60, and 72 hours, for determination of plasma-DA concentrations. Plasma DA-concentrations in the dogs treated with DA plus oxyt–LA (16.75 ± 0.33; 12.85 ± 0.34; 10.29 ± 0.52; 8.55 ± 0.5; 6.89 ± 0.61 and 0.68 ± 0.07µg/ml) were significantly ($p \leq 0.05$) higher PT as compared with 12.95 ±.0.64; 9.66 ± 0.42; 6.46 ± 0.38; 4.56 ± 0.21; 2.83 ± 0.43 and 0.34 ± 0.05 of those treated with DA alone, respectively. AUC last (89.52 ± 5.07 mg/l/hr), AUC total (95.07 ± 5.43 mg/l/hr), AUMC (939.63 ± 109.94 mg.hr^2/l) and $T_{1/2}\alpha$ (2.15 ± 0.25 hr) of the DA -alone group were significantly ($P \leq 0.05$) lower than 141.96 6.60, 151.63 7.30, 1.17 0.19 and 2.00 0.35 of the DA plus oxyt-LA group respectively. Therefore, co-administration of the two drugs could reduce elimination rate of diminazene aceturate thereby enhancing its therapeutic efficacy.

Keywords: Diminazene aceturate, Dogs, Oxytetracycline long acting, Plasma kinectics

Introduction
Pharmacokinetics may be defined as the quantitative study of drug movement in the body (Saganuwan, 2012) [1].

Nigerian indigenous dogs are a breed native to Nigeria and are popularly referred to as "Mongrels or Ekuke" by indigenes. They are long-headed (dolichocephalic) domesticated dogs, with long snout, erect ears and elongated slender body. Their feeding pattern is mainly omnivorous which is as a result of the increased level of domestication (Igado, 2011) [2].

Diminazene aceturate (Berenil) has been the drug of choice for treatment of animal trypanosomosis since 1955. It is an aromatic diamidine consisting of two amidinophenyl moieties linked by a triazene bridge (Peregrine and Mamman, 1993) [26].

Oxytetracycline long acting, a tetracycline derivative obtained from *Streptomyces rimosus*, is a broad-spectrum antibiotic used against a variety of pathogens, including bacteria, mycoplasma, rickettsia, chlamydiae and even some protozoa (Bywater, 1991) [4]. The term "long-acting" implies that the formulation provides a prolonged circulation of antibacterial concentrations of the active agent and shows a commensurate improvement in clinical efficacy (Escudero *et al.* 1994) [5].

Signs of toxicity of diminazene aceturate co-administered with oxytetracycline (long acting) have been reported in dogs (Naude.1970) [6]. Central nervous system (CNS) signs such as tremor, nystagmus and ataxia were observed at lower doses, while higher doses resulted in spasms, uncoordinated movements, vomiting and eventually death in dogs, 2-3 days after intramuscular administration of diminazene aceturate (Giordani *et al*., 2016) [7].

It is worthy of note that heterocyclic compounds (eg. oxytetracycline, diminazene aceturate) have hetero atoms such as nitrogen, sulphur and oxygen which replace carbon in a benzene ring, and could have CNS effects ranging from depression, passing through euphoria to convulsion (Saganuwan, 2017a) [8].

However, short acting oxytetracycline co-administered with diminazene aceturate in dogs did not produce toxic effects (Jennings, 1987) [9]. Hence, there is need to study the effects of oxytetracycline (long acting) on plasma and tissue kinetics of diminazene aceturate in

apparently healthy Nigerian male dogs.

Materials and Method

Fourteen apparently healthy male Nigerian indigenous dogs aged between 4 to 6 months identified morphologically were used in this study. The dogs were presented to the Veterinary Teaching Hospital Michael Okpara University of Agriculture, Umudike, Abia state, Nigeria for clinical assessment of health and vaccination. This study was approved by the University of Agriculture, Makurdi Research and Ethics Committee. The study was conducted according to international guidelines (Wolfensohn and Lloyd, 2013) [10]. Routine medical examination including haemoparasite screening was conducted on all dogs before sampling. Only clinically healthy dogs were sampled. Dogs having haemoparasites and external parasites were excluded. It was ensured that the dogs were calm prior to sampling. Age, sex, body weight, vital parameters and generalized body condition of the animals were assessed. Diminazene aceturate was administered to group one through the left femoral vein at the rate of 3.5mg/kg body weight using 7% solution. Blood samples were obtained from the right femoral vein. Oxytetracycline long acting was administered to group two at the right gluteal muscle deep intramuscularly at the rate of 20mg/kg body weight, ten minutes after administration of diminazene aceturate (3.5mg/kg body weight), blood samples were obtained from the right femoral vein. Blood sample (3 ml) was collected from the femoral vein of each dog using 23G needle and syringe. Fifteen minutes before the drug administration, control blood samples (3 ml) were collected at zero hour and thereafter at 0.25, 0.5, 1, 2, 3, 6, 9, 12, 24, 36, 48, 60, and 72 h and placed in sample bottles containing ethylene diamine tetra-aceturate (EDTA) as an anticoagulant. The blood samples were centrifuged at 2000 rpm for 10 minutes. All the plasma samples collected were stored frozen at -10^{o}C until analyzed. Diminazene aceturate concentration was determined using Perkin Elmer HPLC system with a Flexar UV/VIS Detector (Astriku *et al*., 2002) [11]. The method used for the analysis of plasma solutions before injecting into the HPLC was desbribed by Koshiishi *et al*., (1998) [12]. Retention time for peak of interest was determined. Limit of quantification for diminazene in dog plasma and tissue samples were determined. Dog plasma fortified with standard of diminazene aceturate was used to validate the assay (Da Silva *et al*., 2009) [13]. The plasma concentrations of diminazene aceturate were plotted against time in both groups. Pharmacokinetic parameters were calculated using Pharmacokinetic software (Kinetica 5.0, thermo Fischer scientific) and established pharmacokinetic equations (Aliu *et al*., 1993; Saganuwan, 2012) [14, 1].
Micro (α, β) and macro (A and B) constants were determined by established kinetic graphs.

Statistical Analysis

Data on plasma kinetics were presented in graphs and tables. Plasma concentrations and pharmacokinetic parameters were presented as mean ±standard error of mean (SEM). Test for significance between parameters in the group treated with diminazene aceturate alone and in the group treated with oxytetracycline (LA) was by Student T-test paired. Least significant difference was detected at 5% level (Daniel, 2010) [25].

Results

Validation

The limit of detection of diminazene aceturate was 0.04 µg/ml. The retention time (RT) for peak of interest was from 1.5 min to 2.2 min and limit of quantification (LOQ) of diminazene aceturate in plasma and tissue samples were 0.20 µg/ml and 0.40 µg/g respectively.

Plasma kinetics of diminazene aceturate given alone and in combination with oxytetracycline long acting in apparently healthy Nigerian indigenous dogs

Mean plasma concentration of diminazene aceturate of 20.74±0.61 µg/ml was obtained in the dogs administered diminazene aceturate alone, while 20.10±0.46 µg/ml of diminazene aceturate was obtained in the dogs given a combination of diminazene aceturate and oxytetracycline long acting at 0.25 h post drug administration. Those plasma concentrations thereafter decreased, and at 36 h post drug administration, the concentrations were 0.34±0.0 and 0.68±0.07 µg/ml for diminazene aceturate alone and diminazene aceturate plus oxytetracycline, respectively (Table 1). At 48 h, diminazene aceturate was not detected in the plasma in both groups (Table 1). Nevertheless, the concentration of diminazene aceturate co-administered with oxytetracycline long acting was significantly higher (P<0.05) in comparison with concentration of diminazene aceturate alone (Table 1).

Table 1: Mean plasma diminazene concentrations of apparently healthy Nigerian indigenous dogs administered diminazene aceturate (3.5 mg/kg) alone and its combination with oxytetracycline long acting (20 mg/kg).

Time (hr)	Mean* concentration (µg/ml)	
	Diminazene aceturate	Diminazene aceturate+ oytetracycline LA.
0.25	20.74±.61	20.10±.46
0.5	17.14±.44	18.14±.30
1.0	12.95±.64	16.75±.33[a]
2.0	9.66±0.42	12.85±0.34[a]
3.0	6.46±0.38	10.29±0.52[a]
6.0	4.56±0.21	8.55±0.56[a]
9.0	2.83±0.43	6.89±0.61[a]
12.0	1.34±0.20	2.51±0.29
24.0	0.82±0.14	1.02±0.06
36.0	0.34±0.05	0.68±0.07[a]
48.0	0.00±0.00	0.00±0.00

***** = Mean±SEM based on six observations;
a= p<0.05, paired student's t test.

The pharmacokinetinetic parameters of diminazene aceturate in Nigerian indigenous dogs administered diminazene aceturate alone and diminazene aceturate plus oxytetracycline long acting are shown in Table 2. The pharmacokinetic evaluation of the drug indicated that the data fit a two-compartment open model (Figure 1). The values of elimination rate constant (β), elimination half-life ($T_{1/2\ \beta}$) and mean residence time (MRT) in the dogs administered diminazene aceturate alone (Figure 1) were similar to those of dogs given diminazene plus oxytetracycline long acting (Figure 1). The concentration at time zero (Cp^0 = 34.79±1.74 µg/ml), area under the curve to the last sample point (AUC last = 141.96±6.60 µg/L.h), area under the curve total (AUC total = 151.63±7.30 µg/L.h), area under the moment curve (AUMC = 1515.65±100.83 µg/L.h^2) were significantly (P<0.05) higher in diminazene

aceturate plus oxytetracycline long acting group in comparison with the concentration at time zero (Cp^0 = 26.25± 1.81 µg/ml), area under the curve to the last sample point (AUC last = 89.52±5.07 µg/L.h), area under the curve total (AUC total = 95.07±5.43 µg/L.h) and area under the moment curve (AUMC = 939.63±109.94 µg/L.h^2) of the group treated with diminazene aceturate alone (Figure 1). However, the total body clearance (CL = 0.16±0.01 L/kgh^{-1}), volume of distribution (Vd = 1.31±0.28 L/kg), and distribution rate constant (α = 0.22±0.01 h^{-1}), in the group treated with diminazene aceturate plus oxytetracycline long acting were significantly (P<0.05) lower compared to that of the group treated with diminazene aceturate alone with values of CL (0.12±0.01 L/kgh^{-1}), Vd (1.18±0.01 L/kg) and α (0.35 ±0.04 h^{-1}), respectively in Nigerian indigenous dogs (Figure 1).

Table 2: Pharmacokinetic parameters of diminazene aceturate in apparently healthy Nigerian indigenous dogs treated with diminazene aceturate (3.5 mg/kg) and diminazene aceturate plus oxytetracycline long acting (20 mg/kg).

Parameters	Diminazene aceturate	Diminazene aceturate+oxytetracycline LA
Cp^0 (µg/ml)	26.25±1.81	34.79±1.74[a]
V_C (L/kg)	0.55±0.04	0.51±0.03
A (µg/ml)	18.63±1.63	20.77±0.69
B (µg/ml)	7.42±0.69	14.02±1.65 [a]
AUClast(µg/L.h)	89.52±5.07	141.96±6.60 [a]
AUCtotal (µg/L.h)	95.07±5.43	151.63±7.30[a]
β (h^{-1})	0.11±0.01	0.10±0.01
$T_{1/2}$ β(h)	6.85±0.59	7.01±0.40
MRT (h)	9.81±0.75	9.98±0.43
AUMC (µg/L.h^2)	939.63±109.94	1515.65±100.83[a]
Cl(L/kg.h)	0.16±0.01	0.12±0.01[b]
Vd(L/kg)	1.31±0.28	1.18±0.10[b]
α(h^{-1})	0.35±0.04	0.22±0.01[b]
$T_{1/2}$α(h)	2.15±0.25	3.15±0.13 [a]
K_{12}	0.06±0.02	0.03±0.00
K_{21}	0.18±0.02	0.14±0.01[b]

a= data of diminazene aceturate plus oxytetracycline long acting are significantly higher than those DA treatment alone.
b= data of diminazene aceturate plus oxytetracycline long acting are lower than those DA treatment alone; paired Student tests (p<0.05).

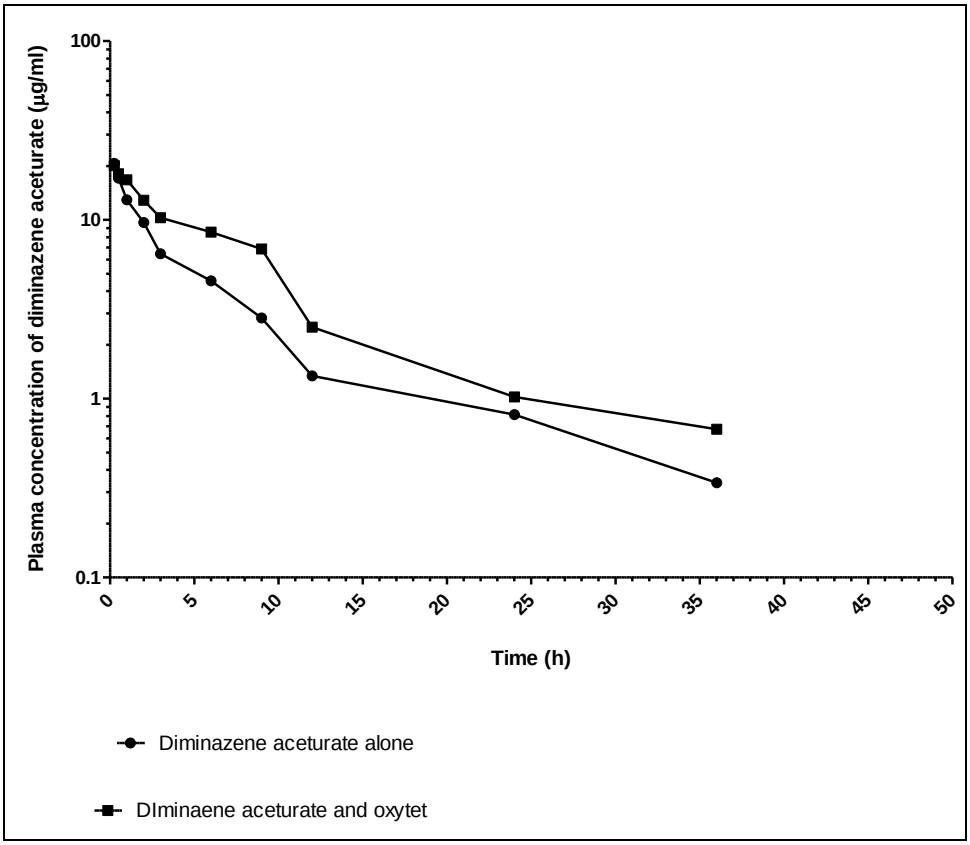

Fig 1: Mean semi-logarithmic plasma concentration curves of intravenous diminazene aceturate (3.5 mg/kg) and diminazene aceturate co-administered with intramuscular oxytetracycline long acting (20 mg/kg) in apparently healthy Nigerian indigenous dogs (n=12).

Discussion and Conclusion

The pharmacokinetic profile of diminazene aceturate in dogs and in its combination with oxytetracycline long acting showed that the drug undergoes a two-compartment model of elimination. This agrees with the report of Mamman *et al.* (1993) [26] and Miller (2005) [17]. The result however, is not

in agreement with the report of Klatt and Hajdu (1976) [18] who reported that diminazene aceturate obeyed one-compartment order of kinetics when co-administered with rolitetracycline. The findings in this study showed that measurable concentrations of diminazene aceturate were present in the plasma of both groups of dogs up to 36 hours post drug-administration. This disagrees with the findings of Onyeyili and Anika (1991) [19], who reported measurable levels (0.2 µg/ml) only at 24 hours post treatment. The observed difference in both studies could be attributed to the assay methods used. Onyeyili and Anika (1991) [19] used colorimetric method, while HPLC method was used for this study which is very sensitive and quantitative. Higher concentration of diminazene aceturate in the group administered diminazene aceturate plus oxytetracycline long acting. This may suggest that oxytetracycline long acting may be interfering with the disposition kinetics of diminazene aceturate. The present findings disagrees with the report indicating that lower concentrations of diminazene aceturate were obtained in the serum of goats administered diminazene plus oxytetracycline long acting as compared to goats administered diminazene aceturate alone (Malgwi, 2021) [20].

The volume of distribution (Vd) relates the drug level in the plasma to the total amount of the drug in the body after attainment of distribution equilibrium (Saganuwan, 2017a) [8]. The higher volume of distribution in dogs given diminazene aceturate alone (1.31± 0.028 L/kg) as compared to its combination with oxytetracycline (1.18±0.10 L/kg) shows that oxytetracycline could decrease volume of distribution of diminazene aceturate in Nigerian indigenous dogs. The greater the volume of distribution, the longer the half-life of elimination ($T_{1/2\beta}$) and the slower will the drug be eliminated from the body (Ahmed, 2015). The volume of distribution obtained in this study is at variance with the findings in dogs administered intramuscular diminazene aceturate (3. 25± 0.007 L/kg) and its combination with secnidazole (Eke *et al*., 2016) [22]. Eke *et al*. (2016) [22] in an earlier study observed higher serum diminazene concentrations in dogs treated with secnidazole plus diminazene aceturate. Young animals of 4-6 months have lower volume of drug distribution (Ginsberg *et al*., 2004) [23]. The concentration at zero time (Cp^o) is the back extrapolated plasma drug concentration and corresponds to the plasma drug concentration immediately after drug administration, if distribution was immediate (Bourne, 2010) [24]. The concentration at time zero (Cp^o) is the sum of the zero time intercepts for distribution (A) and elimination (B). In the present study, the diminazene aceturate plus oxytetracycline long acting increased the Cp^o of diminazene aceturate to 34.79± 1.74 µg/ml as compared to 26.25± 1.81 µg/ml for diminazene aceturate treated dogs. This explained the cause of reported toxicity signs often observe when the two drugs are concurrently used and it implies that the drug combination can be used to treat relapse of trypanosomes infection.

The total body clearance is a reflection of the elimination of drugs from the body (Bourne, 2010) [24] and it is determined by the function of the kidney and liver. The higher the concentration of the drug in plasma, the more the drug is presented for elimination. Thus, clearance is the coefficient of proportionality between plasma drug level and elimination (Bourne, 2010) [24]. Total body clearance of diminazene aceturate was significantly (p<0.05) higher in dogs administered diminazene aceturate alone (0.16±0.01L/kg/h) compared to that of dogs administered diminazene aceturate plus oxytetracycline long acting combination (0.12±0.01 L/kg/h). This may indicate that more drug is presented to the metabolic and excretory organs at any point in the dogs treated with diminazene aceturate alone.

The area under the concentration–time curve (AUC) is the integral of the plasma drug concentration time curve (Bourne, 2010) [24]. It shows the actual exposure of the body to the drug after administration of a drug dose (Bourne, 2010) [24]. AUC is dependent on the rate of elimination and the dose administered and inversely proportional to the total body clearance (Bourne, 2010) [24]. In the present study, treatment with diminazene alone decreased the total AUC (95.07 ±5.43 µg/L.h) in comparison with 151.63±7.30 µg/L.h for diminazene aceturate plus oxytetracycline long acting combination. This may indicate that the exposure of the dogs to the drug is shorter in the group administered diminazene aceturate alone than in the diminazene aceturate and oxytetracycline long acting combination group, because of the higher clearance. Clinically, this implies increased risk of diminazene aceturate toxicity.

Conclusion

Oxytetracycline long acting resulted in significant changes in the pharmacokinetics of diminazene aceturate in apparently healthy Nigerian indigenous male dogs.

Conflict of Interest

Authors have no conflict of interest.

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