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Diminazene aceturate residues in tissues of dogs treated with secnidazole-diminazene aceturate combination and with diminazene aceturate alone

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Abstract

Dimninazene aceturate concentration in plasma and residues in tissues of dogs treated with secnidazole-diminazene aceturate combination and with diminazene aceturate alone was investigated in apparently healthy dogs. Fourteen apparently healthy dogs were randomly assigned to 3 groups. The first group consisted of 6 dogs pre-treated with 100 mg/kg secnidazole (SEC) orally 30 min before administration of 3.5 mg/kg diminazene aceturate (DA) im. The second group consisted of 6 dogs treated with 3.5 mg/kg DA im alone, while the third group had 2 dogs untreated and used to prepare the control tissues and standards. Blood samples were collected at 24, 48 and 72 h post-administration of DA and serum harvested for estimation of DA concentrations in the serum. For estimation of DA residues in tissues, 2 dogs were sacrificed in each group at 240, 360 and 480 h post-administration of the drugs. Ten grams of tissue samples (liver, kidney, brain, heart and skeletal muscle) were collected in triplicate. Intramuscular administration of DA, led to detectable and measurable levels of DA up to 72 h in the serum of both groups of dogs. However, there was no significant difference in the serum concentration of DA in both groups of dogs from 24 – 72 h. The concentration of DA was significantly ($p < 0.05$) higher in the brain of SEC pre-treated dogs at 240 h. In the kidney and liver, DA concentration was significantly ($p < 0.05$) higher in SEC pre-treated dogs at 480 h. There was no significant difference in the DA concentration in the myocardium and skeletal muscles of both groups of dogs. We therefore concluded that DA persists in the tissues of treated dogs beyond 20 days post-treatment and that SEC alters the elimination pattern of DA in SEC pre-treated dogs.

Keywords: Combination, Diminazene aceturate, Dogs, Secnidazole, Tissue residues

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Introduction

Due to the non-effectiveness and toxicity of trypanocidal agents available, drug combinations have been investigated in attempts to either obtain synergism or reduce the severity or incidence of adverse reactions. Onyeyili and Onwualu, (1991) have shown that combination of diminazene aceturate (DA) and eflornithine was curative in the treatment of late-stage *Trypanosoma brucei brucei* infection in rats. However, treatment of dogs infected with relapsing strains of *T. b. brucei* and *T. congolense* with eflornithine and DA combination was not curative (Onyeyili & Anika, 1990). Akpa *et al. (*2008) reported the curative effect of combination of pentamidine isothionate and diminazene aceturate in dogs. Antitrypanosomal effect of secnidazole (SEC) has been reported by Eke *et al.* (2016). More so, the curative effect of SEC-DA combination therapy in rats and dogs experimentally infected with *T. b. brucei* has been reported (Eke, 2016). Various studies on the kinetics of DA in different animal species have

been reported (Kellner *et al*., 1985; Onyeyili & Anika, 1989; El Banna *et al*., 1999; Miller, 2005). Few reports on the tissue residues of DA in animals are available (Onyeyili, 1982; Gilbert, 1983; Kellner, 1985; Onyeyili & Anika, 1991). Klatt & Hajdu, (1976) reported the kinetic of DA in combination with rolitetracycline in cattle. Odika *et al. (*1995) reported that lithium chloride and sucrose increased the concentration of DA in the brain of rats. However, the effect of co-administered drugs with DA on the tissue residues or concentrations of DA in dogs is not available. In this study, the effect of co-administration of SEC and DA on the tissue concentration of DA was investigated. This is important because of the reported greater efficacy of combination therapy of SEC and DA over mono-therapy of DA in dogs (Eke, 2016). This study will throw more light on the possible interactions between SEC and DA in dogs. Also, considering the fact that dog meat is a delicacy in some parts of Nigeria, this study will give information on the possible withdrawal period for dogs treated with SEC-DA combination.

Materials and Methods

Fourteen clinically healthy local dogs of both sexes, between 5 and 10 months old were used for the study. They were kept in a fly-proof dog kennel in separate cages and fed with standard pelletized dry dog food (Dog and Co® for puppies and junior, Adragna, Pet Food Italy). Water was provided *ad libitum*. Prior to the commencement of the study, the dogs were physically examined, and dewormed with prazisam® (fenbendazole, praziquantel and pyrantel pamoate) and screened for the presence of trypanosomes and other hemoprotozoan parasites using Giemsa-stained blood smear. They were vaccinated with distemper, hepatitis, leptospirosis, parainfluenza and parvo-virus polyvalent vaccine (DHLPP) and anti-rabies vaccine (ARV) (Bioveta a.s. Czech Republic). They were acclimatized for 4 weeks before the study commenced. They were separated into three groups. The first group consisting of six dogs were treated with secnidazole (Secwid® May and Baker Nig. PLC) (100 mg/kg) orally, followed 30 min later with diminazene aceturate (Lobazene® France) (3.5 mg/kg) IM. The second group consisting of six dogs were given DA alone at the same dose rate. The third group consisting of two dogs were used to prepare the tissue standards.

A 7% solution of DA in distilled water was administered intramuscularly to the dogs in the left gluteal muscle.

Ethical standards

The ethical conditions governing the use and conduct of experiments with life animals were strictly observed in this study as stipulated by Ward & Elsca (1997) and the experimental protocol was approved by the University of Nigeria Nsukka Senate Committee on Medical and Scientific Ethics.

Sample collection

Control samples were collected from each dog 15 min before drug administration (0 h). Following drug administration, blood samples were collected at 24, 48, and 72 h. Collected samples were centrifuged immediately at 4000 rpm for 5 min, to harvest the serum. For estimation of DA residues in tissues, 2 dogs were sacrificed in each group at 240, 360 and 480 h post-administration of drugs. Ten grams of tissue samples (liver, kidney, brain, heart and skeletal muscle) were collected in triplicate. For preparation of control tissues and standards, the 2 untreated dogs were sacrificed. Tissue samples were placed in plastic bags. The tissues were stored frozen until analyzed. The experimental work area and utensils were cleaned thoroughly after tissue collection from each animal, to prevent contamination.

Analysis of DA concentration in serum and tissues

For the analysis of DA concentration in the serum, the method of Klatt & Hajdu, (1976) was used. One milliliter of serum was added to 1 ml of 10% trichloroacetic acid (TCA). After thorough mixing, the sample was left to stand for 15 min and then centrifuged for 10 min at 4000 rpm. Thereafter 1 ml of clear supernatant solution was added to 1 ml of 1 N HCL in a test tube. This was then diazotized with 0.2 ml of 0.5% sodium nitrite solution. After 3 min, 1 ml of 1% ammonium sulphamate solution was added and the mixture shaken thoroughly. After additional 3 minutes, 1 ml of 0.2% alpha-naphtylethylenediamine solution was added and the developed colour was measured at 540 nm using a UV-spectrophotometer.

For the analysis of tissue residues of DA, the method of Onyeyili & Anika, (1991) was used. In this method, 5 ml of 20% TCA was added to 10 g of tissue and homogenized. The clear supernatant (6 ml) was alkalinized with 0.5 ml 5 m NaOH and extracted 4 times with 5 ml ethylacetate. The organic phase was evaporated to dryness after which 2 ml of 1 M HCL was added and diazotized with 0.2 ml 0.5% sodium nitrite solution. After 3 min, 0.5 ml 1% ammonium sulphamate solution was added and shaken thoroughly. After additional 3 min, 1 ml of 0.4% alpha naphtylethylenediamine was added and the developed color measured at 540 nm with a UV-spectrophotometer.

Statistical analysis

The results were presented as mean ± SD. Data in relation with SEC-DA combination and DA alone

Table 1: Mean diminazene aceturate concentration in serum following intramuscular administration of DA (3.5 mg/kg) and DA plus secnidazole (100 mg/kg)

Time (h)	SEC/DA (DA conc ug/ml) (n=6)	DA (DA conc ug/ml) (n=6)
0	0.00	0.00
24	0.97 ± .02	0.96 ±.01
48	0.94 ± .01	1.04 ± .11
72	0.92 ± .01	0.94 ±. 03

Diminazene aceturate (3.5 mg/kg) was injected 15 min after collection of control sample

Table 2: Mean diminazene aceturate concentration (µg/g) in kidneys of dogs treated with secnidazole-diminazene aceturate combination and of those with diminazene aceturate alone

	DA Concentration (µg/g)	
Time (h)	SEC/DA (n=6)	DA (n=6)
240	2.87 ± 1.10	2.46 ± 0.72
360	2.56 ± 0.06	4.2 ± 0.20*
480	1.92 ± 0.02*	1.81 ± 0.01

*Significant $p < 0.05$

Table 3: Mean diminazene aceturate concentration (µg/g) in the livers of dogs treated with secnidazole-diminazene aceturate combination and of those with diminazene aceturate alone

	DA Concentration (µg/g)	
Time	SEC/DA (n=6)	DA (n=6)
240	2.95 ± 0.79	2.29 ± 0.99
360	4.39 ± 0.09	4.14 ± 0.14
480	2.72 ± 0.02*	2.47 ± 0.07

*Significant $p < 0.05$

were analysed using independent sample T- test. Significance was accepted at $P < 0.05$.

Results

Intramuscular administration of 3.5 mg/kg DA resulted in measurable serum levels of DA for 72 h in both SEC pre-treated dogs and DA alone treated dogs. The mean serum concentrations of DA in both groups did not differ significantly ($p > 0.05$). However, the mean serum concentration of DA was higher in the SEC pre-treated dogs at 24 h, while the dogs treated with DA alone had higher serum concentrations of DA at 48 and 72 h (Table 1).

There was no significant ($p > 0.05$) variation in the DA concentrations in the kidney of both treatment groups at 240 h. Conversely, at 360 h post DA administration, the DA concentration in the kidneys of dogs treated with DA alone was significantly ($p < 0.05$) higher than those of dogs treated with combination of SEC and DA.

At 480 h, the DA concentration in the kidneys of dogs pre-treated with SEC was significantly ($p < 0.05$) higher than that of dogs treated with DA alone (Table 2). The DA concentration was significantly ($p < 0.05$) higher in the liver of SEC pre-treated dogs only at 480 h post-treatment. There was no significant variation between the two groups at 240 and 360 h post-treatment, though higher DA concentrations were found in the livers of dogs pre-treated with SEC at both time periods (Table 3).

There was significantly ($p < 0.05$) higher DA concentration in the brain of SEC pre-treated dogs at 240 h as opposed to that of DA alone. However there was no significant variation afterwards, though DA concentration was higher in the SEC pre-treated dogs at 360 h and by 480 h post-treatment, DA was not detectable in the brain of both groups (Table 4).

Secnidazole did not significantly ($p > 0.05$) alter the DA concentrations in the myocardium of treated dogs. Nevertheless the concentration of DA was higher in the dogs treated with DA alone at 240 h. DA was not detectable in the myocardium at 360 and 480 h in both groups of dogs (Table 5).

Measurable levels of DA were detected in the skeletal muscles of both treatment groups at 240 and 360 h. Although there was no significant difference in the levels of DA in both groups, the DA concentration was higher in dogs pre-treated with SEC at both time periods. At 480 h, DA was not detectable in the skeletal muscles of both groups of dogs (Table 6).

Table 4: Mean diminazene aceturate concentrations (µg/g) in the brains of dogs treated with secnidazole-diminazene aceturate combination and of those with diminazene aceturate alone

	DA Concentration (µg/g)	
Time	SEC/DA	DA
240	3.91 ± 0.91*	2.07 ± 0.07
360	1.68 ± 0.08	1.44 ± 0.44
480	0.00 ± 0.00	0.00 ± 0.00

*Significant $p < 0.05$

Table 5: Mean diminazene aceturate concentrations (µg/g) in the hearts of dogs treated with secnidazole-diminazene aceturate combination and of those with diminazene aceturate alone

	DA Concentration (µg/g)	
Time	SEC/DA	DA
240	1.65 ± 0.20	1.95 ± 0.87
360	0.00 ± 0.00	0.00 ± 0.00
480	0.00 ± 0.00	0.00 ± 0.00

Table 6: Mean diminazene aceturate concentrations (µg/g) in the skeletal muscles of dogs treated with secnidazole-diminazene aceturate combination and of those with diminazene aceturate alone

	DA Concentration (µg/g)	
Time	SEC/DA	DA
240	3.72 ± 0.34	2.39 ± 0.85
360	2.54 ± 0.54	1.99 ± 0.99
480	0.00 ± 0.00	0.00 ± 0.00

Discussion

Our findings showed that measurable levels of DA were detectable in the serum of both groups of dogs up to 72 h post-administration intramuscularly. This disagrees with the findings of Onyeyili & Anika, (1991), who reported measurable levels of (0.2 µg/ml) only at 24 h. The difference in both studies could be attributed to the assay method. Onyeyili & Anika, (1991) used colorimetric method, while in this work UV-spectrophotometric method was used, which is a more sensitive instrument than colorimeter.

Studies on the influence of secnidazole on tissue concentrations of DA showed that DA is readily distributed to various organs and tissues of dogs and persists for up to 20 days post-administration. Highest accumulations of DA were found in the liver and kidney. These findings support the findings of Onyeyili & Anika, (1991) who also found higher DA concentrations in the kidneys and livers of dogs. The reason for higher accumulation of DA in the liver and kidneys could be due to the fact that these are the major organs for metabolism and excretion of drugs (Rang *et al.*, 1996). Diminazene aceturate concentrations in the kidney of SEC pre-treated dogs were higher at 240 and 480 h, while that of dogs treated with DA were higher at 360 h. In the liver, DA concentration was higher in SEC pre-treated dogs at 240, 360 and 480 h. These higher concentrations of DA in the kidneys and livers of dogs pre-treated with SEC could be related to alteration of elimination pattern of DA by SEC. Furthermore higher brain concentrations of DA were observed in dogs pre-treated with SEC at 240 and 360 h. Diminazene was no more detectable in the brain at 480 h in all treated dogs. This finding could suggest that SEC enhances penetration of DA through the blood brain barrier. 5-nitroimidazoles attain high concentrations in the brain (Rang *et al.*, 1996), therefore could act as a transporter of DA into the brain. Clinically this is important in that enhanced accumulation of DA in the brain could lead to elimination of sequestered trypanosomes in the brain. This may explain why relapse of infection was not observed in dogs treated with the SEC-DA combination therapy (Eke, 2016). Diminazene aceturate was only detected in the hearts of treated dogs at 240 h and there was no significant ($p > 0.05$) difference in the concentrations of the two groups, though higher concentrations were found in the heart of dogs treated with DA alone. The concentrations of DA in the hearts of animals in both groups were however, higher than that reported by Onyeyili & Anika, (1991) at same time period. In the skeletal muscles, higher DA concentrations were found in dogs pre-treated with SEC at 240 and 360 h. No DA was detected at 480 h.

We concluded that SEC enhanced accumulation of DA in various tissues of dogs which may enhance the therapeutic efficacy of DA. Our study also showed that DA persists in the tissues of dogs treated with either SEC-DA combination or DA alone up to 480 h (20 days) post-treatment,

though higher concentrations was observed in SEC pre-treated dogs. This should be taken into consideration in estimation of withdrawal period of DA in treated dogs.

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