Sokoto Journal of Veterinary Sciences
(ISSN 1595-093X)
Jatau *et al*. /*Sokoto Journal of Veterinary Sciences* (2010). **8**(1&2):4-8.

Efficacies of diminazene aceturate and isometamidium chloride In *Trypanosoma evansi* experimentally infected rats

ID Jatau*[1], AI Lawal[1], RIS Agbede RIS[1] and EM Abdurrahman[2]

[1]Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.
[2]Department of Pharmacognosy and Drug development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

*Correspondence: Tel.: 08030569756, 08029316789 E-mail: idjatau@abu.edu.ng; mail4abusafiyya@yahoo.co.uk

Abstract
Efficacies of Diminazene aceturate (Berenil^{R}) and Isometamidium chloride (Veridium^{R}) were studied in *Trypanosoma evansi* experimentally infected albino rats. The criteria used for the assessment of the anti-trypanosomal effect of the drugs included the examination of the blood and tissues for *T. evansi* after treatment. Fifty-four albino rats were inoculated with approximately 1.2 x 10^{6} *Trypanosoma evansi* and then randomly grouped into 4 groups of 12 rats each viz groups A, B, C and D. Rats in groups A and B were treated with 3.5 mgkg^{-1}and 7mgkg^{-1} Berenil respectively; groups C and D were treated with 0.5mgkg^{-1} and 1 mgkg^{-1} Veridiuim respectively. The remaining untreated 6 rats (Group E) served as control. Four rats from each of the treatment groups were treated with the drugs on 5, 8, and 11 days post infect ions (dpi). Parasitaemia in the untreated control and the treated rats were monitored using Haematocrit Centrifuge Technique (HCT) twice weekly. Two rats from each group were sacrificed at 45 dpi; visceral organs, brain and testis were collected for impression smears. Berenil was able to clear the parasitaemia in all rats treated with the drug. However relapse of parasitaemia occurred in all the Berenil groups except those treated early at 5 dpi with a dose of 7mgkg^{-1}. Veridium on the other hand was not effective in the elimination of the parasites from circulation in all the groups treated with the drug except those treated early at 1 mgkg^{-1}. Relapse of the parasitaemia however; occurred in all rats in this group within days 11-14 post treatment. With the exception of those treated with Berenil at a dose of 7mgkg^{-1}, trypanosomes were seen in all the rat's organs examined irrespective of the drugs used. It is suggested that the *T. evansi* isolate is resistant to isometamidium chloride at the recommended doses and to low normal dose of diminazene aceturate. However, it is susceptible to high dose of diminazene aceturate when treatment is instituted at early stage of the infection.

Keywords: Diminazene aceturate, efficacy, isometamedium chloride, rat, *Trypanosoma evansi.*

Introduction:
Infection due to *Trypanosoma evansi*, commonly known as "surra" is a very important disease affecting wide range of hosts. Natural infections occur in camels, horses, donkeys and dogs (Al-Rawashdeh *et al*., 2000).Surra is increasingly gaining economic importance in northern parts of Nigeria due to the increasing value of camel as a source of meat and draught animals (Falope, 1991; Mohammed *et al*., 1997).

The control of trypanosomosis continues to rely principally on chemotherapy and chemoprophylaxis using trypanocidal drugs; because no effective vaccine has been developed yet against the disease due to the antigenic variation of trypanosomes, and the absence of coherent environmental friendly and sustainable vector control strategies (ILRAD, 1990). In Africa, the salts of three compounds: diminazene, homidium and isometamidium were relied upon for the control of animal trypanosomosis including surra, because of lack of new animal trypanocides in the region (Holmes *et al*., 2004).

Previous reports have shown that diminazene and isometamidium are therapeutically effective against clinical *T. evansi* infection in camel, cattle, water bufallo and donkeys (Joshi & Singh, 2000). Although these drugs have been used widely, there are often conflicting reports of their efficacies, suggesting that there might be differences in sensitivity amongst isolates of *T. evansi* from different regions.

This work was therefore carried out to study the therapeutic efficacy of diminazene and isometamidium to *T. evansi* in experimentally infected albino rats. As this may assist in predicting the effectiveness of the drugs to different local isolates of *T. evansi* in natural infection.

Materials and methods

Source of the Trypanosoma evansi isolate

The *T. evansi* used in this study was obtained from the blood of naturally infected camels during slaughter at Kano abattoir, Kano State, Nigeria. The blood samples were obtained from severed jugular vein into sterilized Bijou bottles containing salt of ethylene diamine tetra-acetic acid (EDTA), and transported on ice to the Protozoology laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria for analysis. No parasite was observed in the samples using the wet mount and haematocrit centrifugation technique (HCT), but intraperitoneal inoculation of the pooled samples into rats elicited parasitaemia 33 days post inoculation. More rats were sub-inoculated to obtain sufficient inoculums for experimental infection.

Experimental animals

Fifty-four adult albino rats of either sex weighing between 120 to 150g were obtained from the Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria. They were housed in clean cages at room temperature (25°C-30°C) ,fed with pelleted commercial chick mash (PLS feed, Zaria) and water was given *ad libitum*. The animals were screened for presence of blood parasites by standard technique (Schalm *et al*., 1975), and conditioned for 3 weeks before experimental infection.

Experimental infection and groupings

At peak parasitaemia, the donor rats were bled and the pooled blood in EDTA was subsequently expanded with phosphate buffered saline glucose (PSG). The experimental rats were each inoculated intraperitoneally with 0.2ml of the infected blood containing approximately 7 x 10^6 trypanosomes ml^{-1} as estimated using the rapid "marching" method of Herbert and Lumsden, (1976). After the inoculation, the rats were randomly divided into five groups. Group A-D of 12 rats each and group E with 6 rats.

Treatment of experimental animals

At 5, 8 and 11 days posts infection (dpi) four rats each from groups A and B were treated intraperitonealy with 3.5mg/kg and 7mg/kg diminazene aceturate (Berenil®, Hoechst, Germany) respectively, while same number of rats from groups 3 and 4 were similarly treated with 0.5mg/kg and 1mg/kg isometamedium chloride (Veridium®, Ceva Sante Animale, Libourne-France) respectively. Rats in group 5 served as untreated control.

Collection of samples

For the first 3 days post inoculation of the animals, blood sample was collected from each rat via tail vein using capillary tube and then twice weekly for the remaining six weeks of the experiment. At day 45 post infection 2 rats from each group were sacrificed and impression smears were prepared from the brain, lung, heart, liver, spleen, kidney and testes.

Monitoring of Parasitaemia and tissue invasion

Parasitaemia was estimated for each sample using the Haematocrit Centrifugation Technique (Woo, 1969). Organ impression smears were prepared and stained with Giemsa stain as described by Adam *et al*., (1971) to identify the parasite in various tissues.

Results:

Diminazene aceturate administration at 3.5$mgkg^{-1}$ 5 dpi cleared the parasitaemia within 3 days post treatment however, at 17 days post treatment, one of the 4 treated rats relapsed. At a higher dose of 7$mgkg^{-1}$ the parasitaemia were not only cleared within 2-4 days post treatment but there was no relapse during the observation period. Delayed treatment at 8 dpi using the same drug at 3.5$mgkg^{-1}$ and 7$mgkg^{-1}$ also cleared the parasitaemia in the treated rats, but relapses occurred within 10-15 and 20-24 days post treatment respectively (Table 1).

Similarly, delayed treatment with 3.5 $mgkg^{-1}$ diminazene at 11dpi cleared the parasitaemia in 3 rats but, there was relapse in 2 of the treated rats 14 days post treatment. Meanwhile, treatment with 7$mgkg^{-1}$ of the drug the same day cleared the parasitaemia in all the 4 rats treated but with a recurrence of parasitaemia in 2 rats 18 day post treatment (Table 1).

Administration of Isometamidium chloride at 0.5$mgkg^{-1}$ was observed to be ineffective as parasitaemia persisted irrespective of the period of treatment. However, treatment with 1 $mgkg^{-1}$ isometamidium chloride at 5 dpi cleared the parasites from circulation with relapses in all the 4 rats within 11-14 days post treatment, whereas administration of 1$mgkg^{-1}$Isometamidium chloride at either 8 or 11 dpi could not eliminate the parasite from circulation throughout the observation period (Table 2).

Table 1: Response of Kano isolate of *T. evansi* to Diminazene aceturate

Group	Sub-group	No. of Rats	Dose (mg/kg)	No. aparasitaemic after treatment	Relapse	
					No.	Period (dpt*)
A	DA_5	4	3.5	4	1	17
A	DA_8	4	3.5	4	4	10 (in all rats)
A	DA_{11}	4	3.5	3	2	14 (in 2 rats)
B	DA_5	4	7.0	4	0	None (up to 34 dpt)
B	DA_8	4	7.0	4	4	20 – 24 (in all rats)
B	DA_{11}	4	7.0	4	2	17 (in 2 rats)

DA_5 = treated with diminazeneaceturate at day 5 post infection
DA_8 = treated with diminazeneaceturate at day 8 post infection
DA_{11} = treated with diminazeneaceturate at day 11 post infection
***dpt** = days post treatment

Table 2: Response of Kano isolate of *T. evansi* to Isometamedium chloride

Group	Sub-group	No. of Rats	Dose (mg/kg)	No. aparasitaemic after treatment	Relapse	
					No.	Period (dpt*)
C	IS_5	4	0.5	0	0	-
C	IS_8	4	0.5	0	0	-
C	IS_{11}	4	0.5	0	0	-
D	IS_5	4	1	4	4	11 – 14 (in all rats)
D	IS_8	4	1	0	0	-
D	IS_{11}	4	1	0	0	-

IS_5 = treated with isometamedium chloride at day 5 post infection
IS_8 = treated with isometamedium chloride at day 8 post infection
IS_{11} = treated with isometamedium chloride at day 11 post infection
***dpt** = days post treatment

Table 3: Distribution of Kano isolate of T. evansi in tissues of experimentally infected rats following treatment with Diminazine aceturate

Group	Sub-group	Dose (mg/kg)	Brain	Heart	Lung	Liver	Spleen	Kidney	Testes	Blood
A	DA_5	3.5	-	-	-	-	-	+	-	-
B	DA_5	7.0	-	-	-	-	-	-	-	-
A	DA_8	3.5	+	+*	+*	++**	-	-	+	+
B	DA_8	7.0	-	+	+*	+*	++**	-	-	+
A	DA_{11}	3.5	-	+	+**	+**	+	-	-	+
B	DA_{11}	7.0	-	-	+*	+*	+	-	-	+
E			-	-	-	+**	+**	-	-	++

DA_5 = treated with diminazeneaceturate at day 5 post infection
DA_8 = treated with diminazeneaceturate at day 8 post infection
DA_{11} = treated with diminazeneaceturate at day 11 post infection
Ctrl = infected untreated group
+ = few trypanosomes (0-5 / field) seen
++ = many trypanosomes (5-10 / field) seen
*** = few degenerated forms seen
**** = many degenerated forms seen
- = no parasite found

Table 4: Distribution of Kano isolate of T. evansi in tissues of experimentally infected rats following treatment with isometamedium chloride

Group	Sub-group	Dose (mg/kg)	Brain	Heart	Lung	Liver	Spleen	Kidney	Testes	Blood
A	IS_5	0.5	-	-	-	-	-	-	-	-
B	IS_5	1.0	-	-	-	-	-	-	-	-
A	IS_8	0.5	-	-	-	-	-	-	-	-
B	IS_8	1.0	-	-	-	-	-	-	-	-
A	IS_{11}	0.5	-	-	-	-	-	-	-	-
B	IS_{11}	1.0	-	-	-	-	-	-	-	-
E			-	-	-	-	-	-	-	-

IS_5 = treated with isometamedium chloride at day 5 post infection
IS_8 = treated with isometamedium chloride at day 8 post infection
IS_{11} = treated with isometamedium chloride at day 11 post infection
Ctrl = infected untreated group
+ = few trypanosomes (0-5 / field) seen
++ = many trypanosomes (5-10 / field) seen
***** = few degenerated forms seen
****** = many degenerated forms seen
- = no parasite found

The examination of organ impression smears of all the rats in the group treated with 7 $mgkg^{-1}$ diminazene aceturate at 5dpi revealed no trypanosomes, but impression smears of the rats with relapsed parasitaemia in the rest of the treatment groups and control revealed presence of the parasites in various organs (Tables 3 & 4).

Discussion

Based on the conventional method for assessment of the sensitivity of trypanosomes to trypanocidal drugs which involves infecting laboratory rodents experimentally, administering the drug and measuring the level of the parasitaemia (Boid *et al* 1996), isometamedium was ineffective in all the treated rats at the recommended dose. This may indicate some level of resistance of the parasite to this drug. Isometamidium resistant *T. evansi* have previously been reported (Luckins & Dwinger, 2004). However, trypanocidal activity was observed when the drug was administered at higher dose in early infection suggests that isometamedium may be effective against the isolate at a high dose. Isometamidium chloride given above recommended dose ($1mgkg^{-1}$) is toxic to camels (Ali and Hassan, 1986), this could therefore limit the use of the drug in natural *T. evansi* infection in these animals; most especially in the treatment of resistant strains.

Initially, treatment with diminazene aceturate at dose of $3.5mgkg^{-1}$ cleared the parasitaemia, suggesting some degree of drug efficacy. However, the relapse observed after some days of the treatment shows the limitation of this drug against the parasite when used at this dose or when the treatment is delayed. The observed therapeutic efficacy of diminazene in the group treated at the dose of $7mgkg^{-1}$ during early stage of the infection (5dpi) indicates that this drug could be effective in the treatment of *T. evansi* when diagnosis is made early.

It has been suggested that, in relapse cases, the tissue-invasive parasite penetrate privileged sites inaccessible to the drug, from which they emerge into the peripheral circulation, to re-establish the infection (Mamman *et al*., 1994). Jenings *et al*., (1977) established that the longer the duration of *Trypanosoma* infection before treatment, the greater the chances of relapse; this relationship was also observed in this study.

It was evident in this study that *T. evansi* was able to invade all the visceral organs, testis and the brain examined. Previous work had also shown that *T. evansi* was capable of invading these organs (Sudarto *et al* 1990). Similar observation was also been made by Lawal *et al*. (2007) in experimentally *T. brucei* infected rats. The invasive ability of *T. evansi* therefore, seems to be in line with that of the *brucei* group, to which *T. evansi* belongs (Losos, 1980). However despite the fact that the organs enhance multiplication of the parasites, they certainly provide the mechanism for destroying them as evidenced by the presence of numerous degenerated forms. The mononuclear phagocytic system might be responsible for the

attack on the parasites (Morrison, *et al*., 1981). The findings from this study seem to indicate that the rate at which these parasites invade the organsis enhanced by administration of trypanocidal drugs as the parasites are seen more in organs of the treated rats than those of the control rats.

In conclusion, the *T. evansi* isolate is resistant to isometamedium chloride at the recommended doses and to low normal dose ($3.5mgkg^{-1}$) of diminazene aceturate especially when treatment is delayed. However, it is susceptible to high doses ($7mgkg^{-1}$) of diminazene aceturate when treatment is instituted at early stage of the infection. Therefore, for successful treatment of *T. evansi* infection, early diagnosis is very crucial.

Acknowledgements

The laboratory assistance we received from the technical staff of the Protozoology Laboratory Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria is very much appreciated.

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