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Relapse of infection in single and mixed trypanosome infections in rats after diminazene aceturate treatment

Tobias Nnia Egbe-Nwiyi[1]*, Ikechukwu Onyebuchi Igbokwe[1], and Patrick Azubike Onyeyili[2]

[1]*Department of Veterinary Pathology, University of Maiduguri, Maiduguri, Borno State, Nigeria*

[2]*Department of Veterinary Physiology and Pharmacology, University of Maiduguri, Maiduguri, Borno State, Nigeria*

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ABSTRACT

Four groups (A, B, C and D) of 10 rats in each group were used to investigate relapse of infection in single and mixed trypanosome infections. Groups A served as uninfected control, groups B and C were infected with *Trypanosoma brucei* (Wamba strain) and *T. congolense* (Federe strain), respectively, and group D was infected with both species (50% of each species in the infective inoculum). Each infected rat received 1×10^6 trypanosomes by intraperitoneal injection of the inoculum. On day 14 post-infection, when the parasitaemia had reached the peak, infected rats were treated with diminazene aceturate at a dose of 10.5 mg/kg as a single intraperitoneal injection. All rats were aparasitaemic after treatment until days 42 and 49 post-treatment, when relapse of *T. brucei* infection occurred in two rats in each of groups B and D, respectively. All effectively cured rats remained aparasitaemic until the end of experiment on day 91 post-treatment. In conclusion, only *T. brucei* relapsed in 20% of treated cases in both single and mixed infections, whereas no relapse occurred in treated *T. congolense* infection, thus indicating that the interaction of *T. brucei* and *T. congolense* in mixed infections did not affect the curability of *T. congolense* infection.

Key words: chemotherapy, *Trypanosoma brucei*, *Trypanosoma congolense*, diminazene aceturate, mixed infection, rats

Introduction

Diminazene aceturate (Berenil®) is one of the conventional trypanocides for treatment of African trypanosomosis. Resistance of trypanosomes to the drug has been reported

* Contact address:
Dr. Tobias Nnia Egbe-Nwiyi, Department of Veterinary Pathology, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria, Phone: +080 35 642 698; E-mail: tncegbe@hotmail.com; yusufkutigi@yahoo.co.uk

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(ONYEYILI and EGWU, 1995). Although resistance to this drug was reported only with *Trypanosoma brucei* in Nigeria and Uganda, *T. congolense* resistance also occurred in Ethiopia and Zimbabwe (GEERTS and HOLMES, 1998). After treatment with the drug, relapse infections had occurred in *T. brucei*-infected (ONYEYILI and ONWUALU, 1991; EGBE-NWIYI and ANTIA, 1996) and *T. congolense* infected animals (BURUDI et al., 1994; MAMMAN et al., 1994 and 1995).

Natural mixed infections have been reported (JOSHUA and IGE, 1982; KALU et al., 1991) although it is not known whether the interaction of the trypanosome species in mixed infections would affect sensitivity of each species to the drug. Multiple species relapse infections after treatment may occur in the field, but such information seems not to be presently available.

In the present study, relapse of infection after diminazene aceturate treatment was studied in single and mixed trypanosome (*T. brucei* and *T. congolense*) infections in rats.

Materials and methods

Experimental animals. Forty healthy adult albino rats of both sexes weighing between 180-198g obtained from the Department of Pharmacology, University of Jos, Nigeria, were used for the study. They were housed in clean cages at room temperature (30-35 ^{0}C) in a y-proof house, fed commercial diets (ECWA Feeds Ltd. Jos, Nigeria) and water was offered ad libitum. The rats were screened for the presence of blood parasites using wet and Giemsa-stained thin blood films (COLES, 1980) prior to commencement of the experiment.

Trypanosomes. Trypanosoma brucei brucei (Wamba strain) and *T. congolense* (Federe strain) were obtained from the National Institute for Trypanosomosis Research (NITR), Vom, Nigeria. The organisms were maintained separately by serial passages in rats.

Trypanocidal treatment. Diminazene aceturate (Berenil®, Hoechst, Farbwerk AG, Frankfurt am Main, Germany) was diluted to 7% and given as a single intraperitoneal injection at a dose of 10.5 mg/kg to the infected rats.

Experimental design. Four groups (A-D) of 10 rats were used as follows: Group A comprised uninfected controls. Groups B and C were infected with *T. brucei* (1×10^6 trypanosomes), and *T. congolense* (1×10^6 trypanosomes), respectively. Each rat in group D was infected with *T. brucei* (1×10^3 trypanosomes) and *T. congolense* (1×10^3 trypanosomes). The rats were infected with blood from previously infected donors after dilution with phosphate buffered saline solution (pH 7.4). Tail blood was used to determine packed cell volume (PCV) and the level of parasitaemia by the microhaematocrit centrifuge method and haemocytometer method, respectively (COLES, 1980). Infected rats were treated with diminazene aceturate on day 14 post-infection (pi). Thereafter, they were monitored for relapse of infection and full recovery from anaemia after treatment.

Statistical analysis. Obtained data were summarized as means ± standard deviations and analysis of variance (ANOVA). Student's *t*-test was used to compare means (CHATFIELD, 1983).

Results

The mean prepatent periods in groups B, C and D were 5.3 ± 0.8, 7.3 ± 0.7 and 4.5 ± 0.5 days, respectively. The prepatent period in *T. congolense* infected rats was longer ($P<0.05$) than in *T. brucei* infected rats and rats with mixed infections. Those with mixed infections had a shorter prepatent period. The level of parasitaemia increased progressively (Fig. 1a) and was significantly ($P<0.05$) higher in the rats with mixed infections (Group D) than those with single infections (Groups B and C). The infection caused a comparable decrease in PCV in groups B, C and D on day 14 pi (Fig. 1b).

After the trypanocidal treatment on day 14 pi, the PCV began to recover. However,

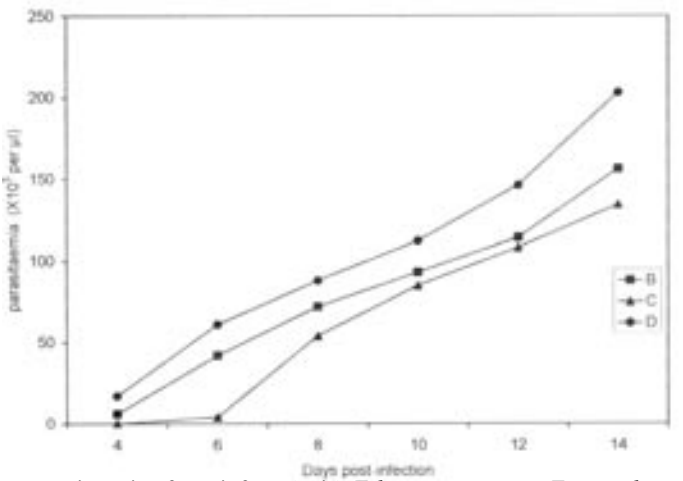

Fig. 1a. Mean parasitaemia of rats infected with *T. brucei* (group B), *T. congolense* (group C) and a combination of *T. brucei* and *T. congolense* (group D)

there was a relapse of infection due to *T. brucei* in groups B and D on days 42 and 49 post-treatment (pt), respectively. Two rats in each of those groups showed relapse. In the effectively cured rats, PCV continued to recover until day 91 pt (end of experiment) when the mean PCV value of the groups were almost comparable with the pre-infection mean PCV (Fig. 1c). At this time, the relapsed cases had a mean PCV of 20.8 ± 0.4%, compared to 44.6 ± 11.3% at day 14 pi.

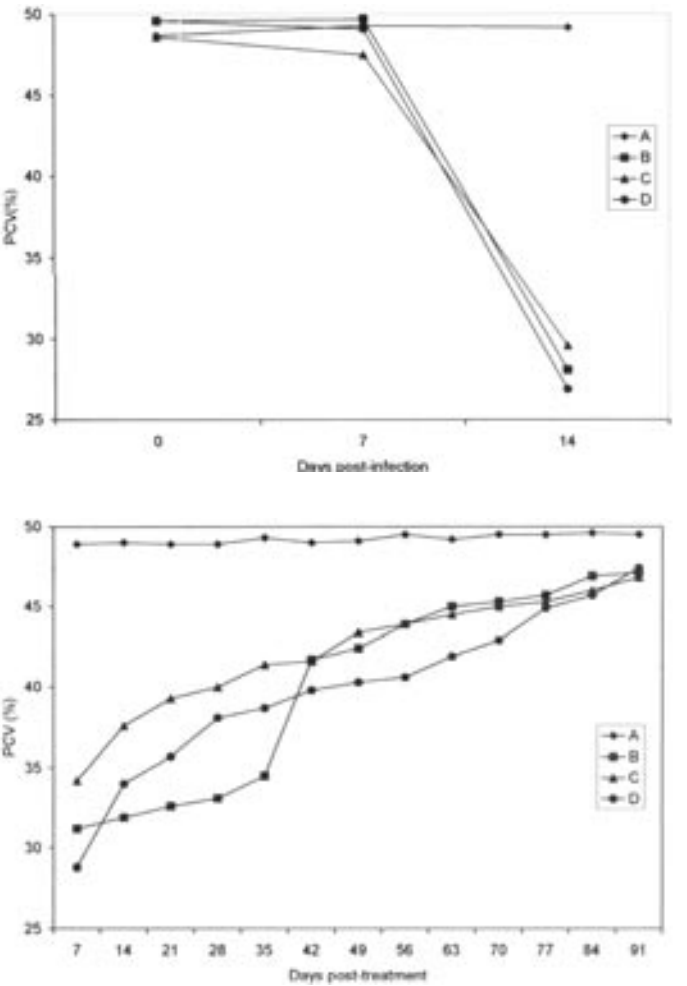

Fig. 1b and c. Mean packed cell volume (PCV%) of control rats (group A) and rats infected with *T. brucei* (group B), *T. congolense* (group C) and a combination of *T. brucei* and *T. congolense* (group D) and responses of the various groups (B, C and D) to treatment with diminazene aceturate

Discussion

Each infected rat in all groups received the same number of trypanosomes in the infective inoculum. This was to eliminate the possible in uence of infective dose on the prepatent period and subsequent parasitaemia (MURRAY and DEXTER, 1988). Prepatent periods were shorter in *T. brucei* than *T. congolense* infections, as earlier reported (IGBOKWE and NWOSU, 1997). Thus, *T. brucei* would appear in circulation earlier than *T. congolense* in mixed infections. The mixed infections showed higher parasitaemia than the single infections, an observation supported by reports that natural mixed infections were often more severe (LOSOS, 1986).

When the infections were treated at the peak of parasitaemia (14 days pi) with diminazene aceturate at a dose of 10.5mg/kg, *T. brucei* was cured in only 80% of cases in the single and mixed infections, while *T. congolense* was cured in all cases. Cured animals were observed for 91 days, beyond the 60 days recommended for evaluation of drug sensitivity in trypanosome chemotherapy (GEERTS and HOLMES, 1998). It appeared that *T. congolense* strain was not resistant to diminazene aceturate. This is consistent with the lack of reports of diminazene aceturate -resistant *T. congolense* in Nigeria (GEERTS and HOLMES, 1998). However, relapse of *T. brucei* infection occurred in the same proportion in groups with single and mixed infections, and similar relapses has been reported by other authors in only single infections (ONYEYILI and ONWUALU, 1991; EGBE-NWIYI and ANTIA, 1996).

After treatment the animals showed aparasitaemia and apparent gradual recovery of PCV, but by days 42-49 pt, the trypanosomes broke into circulation again in cases with therapeutic failure. The subsequent parasitaemic relapse prevented the recovery of PCV in the uncured cases, but PCV fully recovered in cured cases. The relapse of *T. brucei* infection might have originated from privileged sites inaccessible to the drug (MAMMAN et al., 1994; ONYEYILI and EGWU, 1995) where the trypanosomes would not have been exposed to curative concentrations of drug. Since *T. brucei* was more tissue-invasive than *T. congolense* it was speculated that tissue damage by *T. brucei* would have enhanced access of *T. congolense* into drug-inaccessible sites to encourage its subsequent relapse. The interaction of *T. brucei* and *T. congolense* in the mixed infections did not seem to in uence the drug sensitivity of *T. congolense*. The trypanosomes from the relapse infection in rats with mixed infections were studied in subsequent passages in naïve rats. No *T. congolense* sub-population was identified (EGBE-NWIYI, 2002).

Although 3.5mg/kg is the recommended curative dose of diminazene aceturate in trypanosome infections (CUNNINGHAM and GRAINNAGE, 1965), higher doses (7-10.5 mg/kg) have been used (KAGGWA et al., 1988; ONYEYILI and ONWUALU, 1991; KALU, 1995; EGBE-NWIYI and ANTIA, 1996) for anticipated improved efficacy. Since underdosing enhances the chance of development of drug resistance (GEERTS and HOLMES, 1998),

increasing dosage appears to be a corollary for avoidance of such drug resistance. Relapse of infection occurred at the higher dose used in this study to buttress the recommendation that higher doses should not be used to eliminate relapse of infection (EGBE-NWIYI et al., 2003).

In conclusion, treatment of single and mixed infections of *T. brucei* and *T. congolense* in rats with diminazene aceturate at 10.5mg/kg led to relapse of infection of *T. brucei* (20% of cases) in both single and mixed infections, although *T. congolense* infected animals were effectively cured in all cases. Therefore, interaction of both species in mixed infections did not encourage relapse of *T. congolense* infection, nor did it affect the frequency of relapse of *T. brucei* infection.

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SAŽETAK

Istraživanje je provedeno na 4 skupine (A, B, C, D) po 10 štakora invadiranih jednom ili dvjema vrstama tripanosoma. Skupina A predstavljala je neinvadiranu kontrolu. Skupina B bila je invadirana vrstom *Trypanosoma brucei* (soj Wamba), a skupina C vrstom *Trypanosoma congolense* (soj Federe). Skupina D invadirana je objema vrstama (50% svake vrste u inokulu). Svaki štakor invadiran je s $1x10^6$ tripanosoma ubrizgavanjem u peritonejsku šupljinu. Četrnaestoga dana nakon invazije, na vrhuncu parazitemije, štakori su liječeni ubrizgavanjem 10,5 mg/kg diminazen-aceturata. Prisutnost tripanosoma u krvi nije bila ustanovljena sve do 42. i 49. dana nakon

liječenja, kada je u dvaju štakora iz skupina B i D ustanovljena vrsta *T. brucei*. Parazitemija nije bila dokazana u ostalih štakora sve do završetka pokusa 91. dana nakon invazije. U zaključku se navodi da se invazija vrstom *T. brucei* povratila u 20% invadiranih štakora. Povratak invazije nije zabilježen u skupini invadiranoj vrstom *T. congolense*, što govori da međusobno djelovanje vrsta *T. brucei* i *T. congolense* u mješovitoj invaziji ne utječe na izlječenje invazije praživotinjom *T. congolense*.

Ključne riječi: kemoterapija, *Trypanosoma brucei*, *Trypanosoma congolense*, diminazen-aceturat, mješovita invazija, štakori