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SHORT PAPER

Co-administration of Na-EDTA and Diminazene Aceturate (DA) to Mice Infected with DA-resistant *Trypanosoma brucei*

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Summary

This study investigated the effects of co-administration of Na-EDTA and diminazene aceturate (DA) on the level of parasitaemia (LOP), parasite clearance, packed cell volume (PCV) and post-infection survival time (PIST) in mice infected with DA-resistant *Trypanosoma brucei*. Five groups of 10 mice were treated as follows: infected and treated with Na-EDTA+DA; infected and treated with DA alone; infected and treated with Na-EDTA alone; infected–untreated; and uninfected–untreated. The co-administration of Na-EDTA and DA led to reduced LOP and improvements in PCV ($P<0.05$), as compared with treatment with DA alone. Mice treated with Na-EDTA+DA had a marginally ($P>0.05$) higher PIST than did mice treated with DA alone. Comparison of the group given Na-EDTA alone with the infected-untreated group showed that the former group had a significantly lower ($P<0.01$) LOP, improved PCV ($P<0.05$) and higher ($P<0.01$) PIST. It was concluded that the co-administration of Na-EDTA and DA led to a slight potentiation of DA in the treatment of mice infected with DA-resistant *T. brucei*, and that the administration of Na-EDTA alone significantly enhanced the resistance of the infected mice.

Keywords: diminazene aceturate; mouse; Na-EDTA; parasitic infection; *Trypanosoma brucei*

Introduction

Animal trypanosomosis is an economically important disease of livestock in tsetse-infested areas of Africa (PAAT, 2001; Shaw, 2004). Infection is associated with irregular fever, anaemia, emaciation or weight loss, impairment of immune function, reproductive disorders, and death if the animals are not treated (Horst, 1996; Taylor and Authie, 2004). Chemotherapy with trypanocides is the most important method of control (PAAT, 2001; Holmes *et al.*, 2004). Over the years, treatment of animal trypanosomosis relied mainly on three drugs—diminazene aceturate, homidium bromide (or chloride) and isomethamidium (Leach and Roberts, 1981; Holmes *et al.*, 2004). Of these three, diminazene aceturate (DA) is the most widely used because of (1) its comparatively high therapeutic index, (2) its activity against trypanosomes that are resistant to other trypanocides, and (3) the low incidence of resistance to it (Peregrine and Mamman, 1993; Holmes *et al.*, 2004; Van den Bossche and Doran, 2004). With the increasing incidence of drug resistance and the non-development or unavailability of new trypanocides, most current research efforts are directed towards making optimal use of the older drugs by combining them with enhancing agents (Geerts and Holmes, 1998; Holmes *et al.*, 2004).

Disodium ethylene diamine tetracetic acid (Na-EDTA) has been used for purposes such as the potentiation of antimicrobial activity of antibiotics, chelation therapy, detoxification and neutralization of poisons (Wooley and Jones, 1983; Wooley *et al.*, 1983; Lambert *et al.*, 2004; Hininger *et al.*, 2005; Lamas and Hussein, 2006). The ability of Na-EDTA to potentiate some antimicrobial agents is dependent upon its effects on

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microbial cell membranes and ribosomes (Leive, 1968; Yuan and Shen, 1975; Wooley and Jones, 1983). Its potentiating activity appears to be particularly significant with those antimicrobials that act on the cell membrane or cell wall and ribosomes, and those that inhibit DNA and protein synthesis (Wooley and Jones, 1983; Wooley *et al.*, 1983; Lambert *et al.*, 2004).

The trypanocidal activity of diminazene aceturate (DA) is due mainly to its ability to inhibit kinetoplast DNA replication and cause alterations in ribosomes, cytoplasmic membranes and amino-acid transport mechanisms (MacAdams and Williamson, 1972; Newton, 1972; Peregrine and Mamman, 1993). The purpose of the present study was to investigate the possible potentiation of DA activity by Na-EDTA in the treatment of mice experimentally infected with DA-resistant *Trypanosoma brucei*.

Materials and Methods

Fifty adult male albino mice aged 12 weeks were used, having been bred at the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The mice were randomly divided into five groups (A–E) of 10, for treatment as follows: infection and treatment with Na-EDTA+DA (group A); infection and treatment with DA alone (group B); infection and treatment with Na-EDTA alone (group C); infection without treatment (controls; group D); no infection or treatment (controls; group E). Individual mice in each group were given indelible identification marks. The mice were caged in groups in a fly-proof animal house and maintained on pelleted commercial feed (Vital Feeds Ltd., Jos, Nigeria) and drinking water *ad libitum*. The experiment followed the University of Nigeria guidelines for the use of laboratory animals.

The trypanosome strain used to infect the mice was the Faculty of Veterinary Medicine-University of Nigeria (FVM-UNN) *T. brucei* stock strain, maintained in albino rats. This strain was originally isolated from a clinically infected dog at the University of Nigeria Veterinary Teaching Hospital, Nsukka, and characterized as being DA-resistant by the standardized method for testing trypanocidal drug resistance (Eisler *et al.*, 2001). The mice in groups A–D were each infected by the intraperitoneal injection of a saline suspension of 1.5×10^5 *T. brucei* in a dose volume of 0.1 ml. Uninfected control mice (group E) were each given a similar injection of normal saline.

Baseline values for the packed cell volume (PCV) of the mice were determined by the microhaematocrit method on the day before the mice in groups A–D were infected ("day 0"). Thereafter, PCV determinations were carried out at 4-day intervals throughout the experiment. After infection, parasitaemia was assessed at 2-day intervals by wet blood film and haematocrit methods (Herbert and Lumsden, 1976; Murray *et al.*, 1977). On day 12 post-infection (PI), when the level of parasitaemia (LOP) was high, the mice in groups A and B were treated intramuscularly with Nozomil® (a brand of diminazene aceturate [Kepro BV, Barneveld, Holland]) 7.0 mg/kg body weight. Mice in group A were also given an intraperitoneal injection of Na-EDTA (BDH Chemicals Ltd., Poole, England) 70 mg/kg body weight/day on days 12, 13, 14, 15 and 16 PI, this daily dose being the maximum recommended (USFDA, 2005). Mice in group C were treated for 5 days with Na-EDTA alone, as above, while mice in group D (controls) remained untreated. After the treatments, the LOP and PCV continued to be assessed at the specified intervals, and the post-infection survival time (PIST) was recorded for each mouse at the time of its death.

Data on the LOP, PCV and PIST were subjected to analysis of variance (ANOVA), expressed as means ± standard error, and presented as line graphs.

Results

Trypanosomes were detected in the blood of most mice (6–8 per group of 10) of groups A–D by day 4 PI, and by day 6 all the infected mice had detectable trypanosomes in the blood. The LOP increased progressively in all the infected groups until after treatment with DA on day 12 PI (Fig. 1). On days 14 and 16 PI, the LOP of the group A mice (treated with Na-EDTA+DA) and group B mice (treated with DA alone) fell dramatically and on days 18, 20 and 22 PI no trypanosomes were detected in their blood (Fig. 1). From day 14 PI onwards up to the end of the experiment, the mean LOP in groups A and B mice was significantly lower ($P < 0.01$) than that of groups C (infected and treated with Na-EDTA alone) and D (infected–untreated group). The mean LOP of group A mice was lower than that of group B throughout the experiment, but the difference were not significant ($P > 0.05$) from day 14 to day 26 PI (Fig. 1).

After DA treatment had cleared trypanosomes from the blood of group A and B mice, a relapse of the infection was observed, first in two group B mice on day 24 PI, and later (day 26 PI) in two group A mice and five group B mice. On day 28 PI, trypanosomes were found in the blood of almost all group A and B mice. The mean LOP then increased progressively in both groups; nonetheless, it was significantly lower ($P < 0.05$) in group A than in group B mice from day 28 PI until day 36 PI, at which time the assessment of LOP terminated (Fig. 1).

In comparing the infected group treated with Na-EDTA alone (group C) with the infected - untreated group (group D), it was observed that from day 14 PI