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Original Research Article

Anti-trypanosomal activity of secnidazole in vitro and in vivo

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Abstract

Purpose: *To evaluate the anti-trypanosomal effect of secnidazole (SEC) in vitro and in vivo.*
Methods: *The dose-response effect of SEC in Trypanosoma b. brucei infected rats was evaluated in five groups of rats (n = 5). Group A was infected but untreated; B was given diminazene aceturate (DA) (3.5 mg/kg) intraperitoneally, while groups C, D and E received orally 400, 800 and 1600 mg/kg of SEC, respectively. The effect of a combination therapy of SEC and DA was studied in 7 groups of rats (n = 5). Group 1 was infected but untreated; groups 2 – 7 were treated with DA (3.5 mg/kg), while groups 3 – 7 received in addition to DA, increasing double doses of SEC (50 – 800 mg/kg).*
Results: *The MIC of SEC and DA were 1.4 and 0.0021 mg/ml respectively. SEC dose-dependently and significantly ($p < 0.05$) lowered parasitemia from day 2 post-treatment (PT) compared with infected untreated rats. Parasitemia was cleared 3 days PT in all combination groups and 5 days PT in DA group. Relapse of infection occurred in Group 2, 13 days PT, and 16 and 27 days PT in Groups 3 and 4 respectively. There was no relapse of infection in Groups 5 – 7 up to 70 days PT.*
Conclusion: *These results suggest that SEC possesses some degree of anti-trypanosomal effect, and that combination therapy of SEC and DA was superior to DA alone.*

Keywords: *Trypanosoma, Secnidazole, Diminazene aceturate, Anti-trypanosomal, Combination therapy*

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INTRODUCTION

Trypanosomiasis is a complex debilitating protozoan disease of both man and animal [1]. Animal trypanosomiasis is a disease complex caused by *Trypanosoma congolense, T. vivax*, or *T. brucei brucei*, or mixed infection of these trypanosomes and transmitted by the tsetse-fly. The disease is characterized by intermittent fever, anemia, occasional diarrhea, and if untreated leads to the death of the infected animal [2]. The major chemotherapeutic agent of animal trypanosomiasis is diminazene aceturate (DA) [3]. Diminazene aceturate also known as 4, 4' - (1-triazene-1, 3 - diaceturate) is indicated for prophylaxis and treatment of trypanosomiasis in cattle, dogs, goats, sheep and swine [4]. DA is an RNA and DNA binder and is antibacterial and antiprotozoal in action [5].

The major setback in the use of DA is relapse infection, drug resistance and toxicity. Relapse infection occurs as a result of sequestration of trypanosomes in organs and tissues like the

brain where DA does not accumulate in therapeutic concentrations [6-8]. Therefore there is need for identification of agents that can improve treatment outcomes and eliminate relapse infection. Some 5-nitroimidazoles like fexinidazole and benznidazole have been used in the treatment of some human forms of trypanosomiasis like Chagas diseases in South America [9,10]. Chagas disease is a human form of trypanosomiasis in South America caused by *Trypanosoma cruzi*. 5–nitroimidazoles are well distributed and accumulate in sufficient amounts in the brain [10,11]. This is an advantage in the treatment of trypanosomiasis.

Secnidazole (SEC) is 1-(2-methyl-5-nitro-1-imidazol-1-yl) propan-2-ol [12]. The antiprotozoal and antiamoebic activities of SEC are due to reduction of the nitro group of nitroimidazole by ferrodoxin [13]. SEC is completely absorbed after oral administration. 5-nitroimidazoles share a common spectrum of activity against anaerobic micro-organisms and they appear particularly effective in the treatment of amoebiasis, giardiasis, trichomoniasis and bacterial vaginiasis [14]. Secnidazole is safe and well tolerated. Its single dose treatments are widely used for amoebiasis, giardiasis, trichomoniasis and genitourinary infections [14]. The high efficacy of SEC in the treatment of these protozoal diseases necessitated investigation into wider application of this drug specifically in the treatment of *T. b. brucei* infection in animals. To the best of our knowledge, there has not been any report on the anti-trypanosomal effect of SEC.

Therefore the objective of this study was to investigate possible anti-trypanosomal effects of SEC *in vitro* and *in vivo*. Possible chemotherapeutic synergy between SEC and DA was also investigated as a combination therapy.

EXPERIMENTAL

Trypanosomes

T. brucei brucei used in this study was originally isolated from a mongrel dog presented at the Veterinary Teaching Hospital University of Nigeria Nsukka. Morphological identification was by standard procedures [15]. The parasites were maintained in rats from which experimental animals were infected.

Animals

Sixty albino rats were used in this study. They were housed in stainless steel cages. Water and feed were provided *ad libitum.* They were allowed to acclimatize for 1 week before each study. The animal experimental protocol was approved by the Experimental Animal Ethics Committee of the Faculty of Veterinary medicine, University of Nigeria, Nsukka and in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC).

In vitro anti-trypanosomal effect of SEC

This experiment was performed using standard protocol for *in vitro* anti-trypanosomal assay [16, 17]. Aqueous suspension (100 µl) of SEC concentration (125 mg/ml) in distilled water was pipetted into microtiter wells. Serial double dilutions of the drug suspension were prepared in the microwells to obtain a final volume of 50 µl in each well. Forty five (45) µl of trypanosome-infected blood containing about 225,000 trypanosomes suspended in phosphate saline glucose (PSG) as nutrient medium was added to each well containing the SEC suspension and the final concentration of SEC in each well was determined. The same procedure was repeated for DA. As control, 50 µl of PSG was pipetted into another well. The set up was incubated for 1 h at 37 ^{o}C.

For assessment of anti-trypanosomal activity, 5 µl of each sample was taken at 10 min intervals, and placed on a microscope slide covered with a cover slip and viewed under the microscope for trypanosome motility. Total inhibition of trypanosome motility was considered trypanocidal. The minimum drug concentration that totally inhibited trypanosome motility within 1 h was taken as the minimum inhibitory concentration (MIC) of the drug.

Infection of rats

Parasitemia was estimated using the rapid matching technique [18]. All rats were infected intraperitoneally (i.p.) using 1 x 10^6 *T. b. brucei* suspended in 0.2 ml of phosphate buffered saline (PBS). The rats were monitored daily for onset of parasitemia using wet mount.

Dose-response effect of SEC in *T. b. brucei*-infected rats

Twenty five rats randomly assigned to 5 groups (A – E) of 5 rats each were used in this experiment. They were grouped as follows: A: infected untreated, B: infected and treated once with DA (3.5 mg/kg) IP; groups C, D and E were infected and treated orally with 400, 800 and 1600 mg/kg SEC respectively for six consecutive days. Parasitemia was monitored daily using the

rapid matching technique [18]. Treatment started on day 5 post-infection (PI). Changes in parasitemia were recorded and daily mean parasitemia was calculated for each group.

Effect of combination therapy of SEC and DA on parasitemia in *T. b. brucei*-infected rats

Thirty-five rats randomly assigned to 7 groups of five rats per group were used. They were grouped as follows: 1: infected untreated; 2 - 7: infected and treated with DA (3.5 mg/kg) IP once; 3 - 7: infected and treated in addition with DA, 50, 100, 200, 400 and 800 mg/kg SEC orally respectively for six consecutive days. Parasitemia was established 5 days post-infection and treatment started on day 8 post-infection. The following parameters were monitored: daily parasitemia, time of clearance of parasitemia and time of relapse of infection. The animals were monitored for 70 days post-treatment for relapse infection. Groups that did not relapse by day 70 were considered cured.

Statistical analysis

The data collected are presented as mean ± SEM. Analyses of data on parasitemia were done using repeated measure ANOVA. Variant means were separated using least significant difference (LSD). Significance was accepted at $p < 0.05$.

RESULTS

***In vitro* trypanocidal effects of secnidazole and diminazene aceturate**

SEC at concentrations of 2.8 and 1.4 mg/ml eliminated trypanosome motility within 10 min of incubation. Lower concentrations of SEC had no visible effect on trypanosome motility. Elimination of trypanosome motility also occurred within the same period for DA concentrations of 0.267 to 0.0041 mg/ml. DA at 0.0021 mg/ml eliminated trypanosome motility 20 min post-incubation while the controls (phosphate saline glucose) had no visible effect on the trypanosome parasites (Table 1).

Dose-response effect of SEC on parasitemia in *T. brucei brucei*- infected rats

There was no significant ($p > 0.05$) variation in the levels of parasitemia on day 5 post-infection (PI) in all the groups. However, from day 6 PI to the end of the experiment, all treated rats showed significantly ($p < 0.05$) lower levels of parasitemia than the infected untreated group except for the SEC (400 mg/kg) on days 7 and 8 PI. There was no significant variation in parasitemia between the SEC and DA treated groups on days 5, 6 and 7 PI; nevertheless parasitemia was significantly ($p < 0.05$) lower in the DA treated group on days 8 and 9 and by day 10 PI, the parasites were completely cleared from the blood in this group as opposed to SEC treated groups (Figure 1).

Table 1: *In vitro* antitrypanosomal effects of SEC and DA

Secnidazole concentrations (mg/ml)	**Incubation time (min)**					
	10	20	30	40	50	60
2.8	-					
1.4	-					
0.7	+	+	+	+	+	+
Phosphate saline glucose	+	+	+	+	+	+
Diminazene aceturate concentration (mg/ml)						
0.264	-					
0.134	-					
0.067	-					
0.033	-					
0.017	-					
0.0084	-					
0.0041	-					
0.0021	+	-				
0.001	+	+	+	+	+	+
Phosphate saline glucose	+	+	+	+	+	+

(+) = parasites were motile; (-) = parasites were immotile

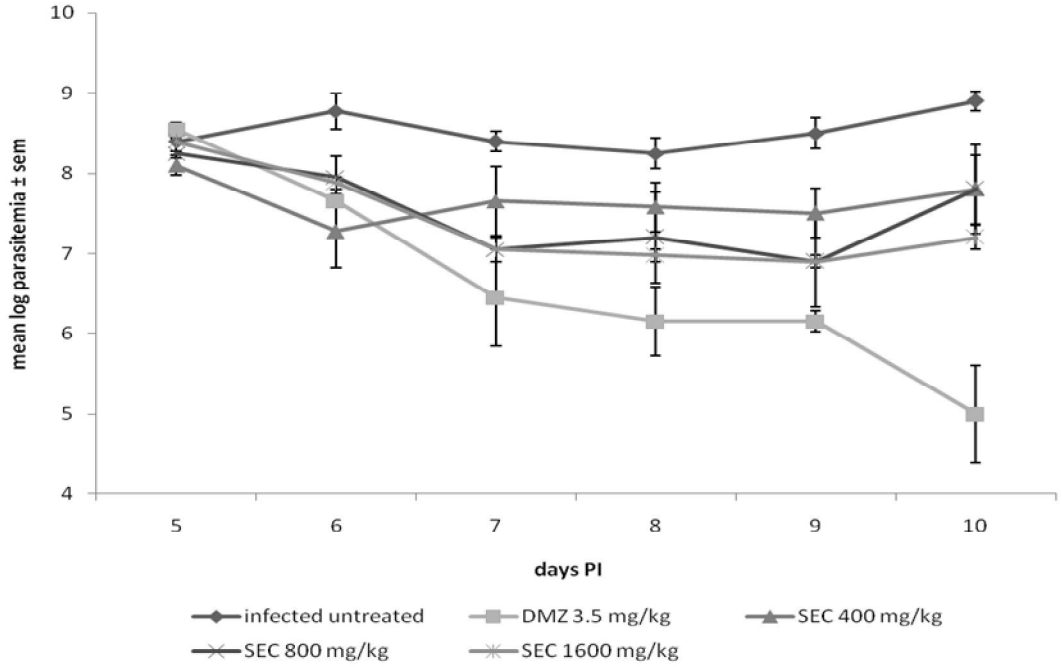

Figure 1: Effect of SEC on parasitemia in rats infected with *T. brucei brucei*. ***Note:*** Log parasitemia below 5.4 shows undetectable parasitemia

Effect of combination therapy of sec on parasitemia in *T. b. brucei* infected rats

There was no significant variation in parasitemia on day 8. However, on day 9, groups 4 and 5 had significantly ($p < 0.05$) lower parasitemia than groups 1 and 2. In addition, group 6 had significantly ($p < 0.05$) lower parasitemia than group 3. From day 10 onwards all the treated groups had significantly ($p < 0.05$) lower parasitemia than the untreated. Relapse of infection occurred in group 2 on day 21, in group 3 on day 24, and on day 35 PI for group 4. By day 20 PI all untreated rats had died. There was no relapse of infection in groups 5, 6 and 7 up to day 70 when the experiment was terminated (Figure. 2).

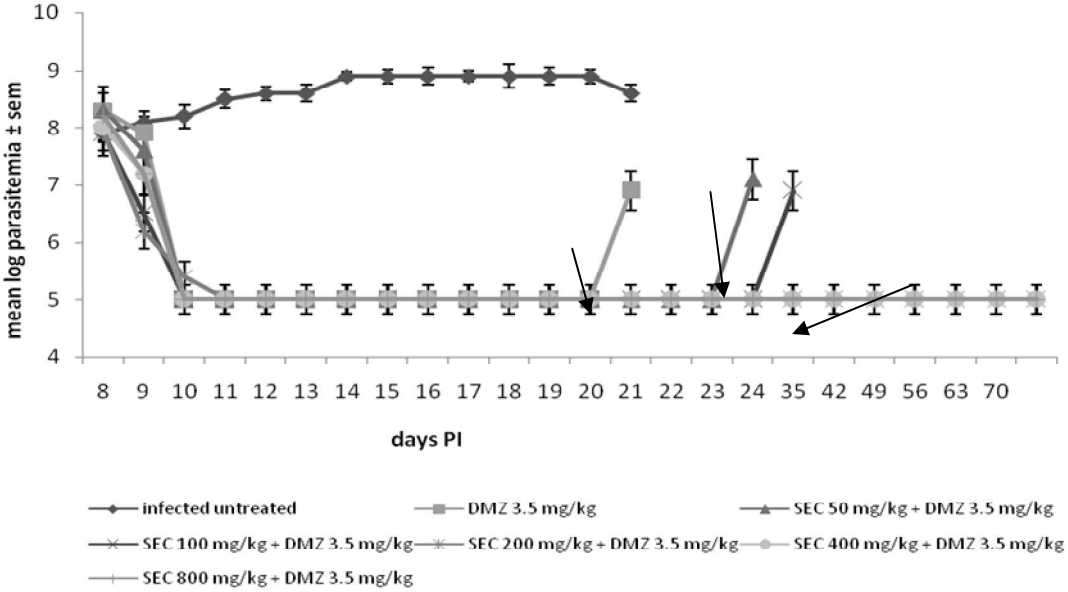

Figure 2: Effects of combination therapy of SEC and DA on parasitemia in rats infected with *T. brucei brucei.* ***Note:*** Mean log parasitemia below 5.4 indicates undetectable parasitemia.The arrows show points of relapse (reappearance of parasites in the blood after initial disappearance following treatment). Data collection on parasitemia was terminated as soon as relapse was recorded for any group. Animals that did not relapse by day 70 PI are assumed to be cured

DISCUSSION

In the present study, SEC showed promising *in vitro* trypanocidal efficacy at concentrations of 2.8 and 1.4 mg/ml. In rats the effect of SEC was dose-dependent with significant anti-trypanosomal effect. In combination with DA, earlier clearance of parasite was achieved and relapse of infection was prevented at higher doses of SEC. The MIC of SEC was very high when compared with DA. This may suggest that higher doses of SEC would be required to produce anti-trypanosomal effects *in vivo*. The SEC-induced death of trypanosomes shows that trypanosomes are sensitive to the antimicrobial effects of SEC. However, it seems that for optimum drug uptake by trypanosome, SEC has to be available in high concentrations. Furthermore the effect of SEC did not appear to be time-dependent; rather it was concentration-dependent. Therefore further investigation of SEC for *in vivo* effect was necessary.

The dose-response effect of SEC in rats showed that SEC has significant anti-trypanosomal effect which was dose dependent. However SEC was not able to completely eliminate trypanosomes at all doses used within the period of the study. All SEC-treated groups maintained a low grade steady state parasitemia throughout the period of treatment. However by day 10 PI, there was a surge of parasitemia in all SEC-treated groups, while parasitemia cleared completely in the DA-treated group. The anti-trypanosomal effect of SEC as observed in this study appears to be trypanostatic in action and may require a functional immune system for total clearance of parasitemia. It is known that trypanosomiasis cause severe immunosuppression [19]. This may explain the persistent and subsequent surge of parasitemia on day 10 PI. This resurgence of parasitemia could be due to progressive immunosuppression by trypanosomes and increased multiplication of the trypanosomes. Antiprotozoal activity of SEC is by reduction of nitro group of nitroimidazole by ferrodoxin.

The nitro group of secnidazole is reduced to intermediate compounds in microorganisms which cause cytotoxicity by damaging DNA [13]. It is not clear whether or not this mechanism is involved in the anti-trypanosomal effect of SEC. Howver, it is clear from this study that trypanosomes are sensitive to some antimicrobial effects of SEC. Thus for full exploitation of the observed antitrypanosomal effects of SEC, further studies in the area of combination therapy with other potent trypanocidal drugs like DA was necessary.

Combination therapies are used either to obtain synergism or reduce incidence of drug resistance and toxicity or reduce the incidence and severity of adverse drug reactions [6]. The combined therapy of SEC and DA in rats infected with *T. b. brucei* was more effective than mono-therapy of DA. There was faster clearance of parasitemia in all the combined therapy groups than the DA group. Parasitemia cleared in all the combined treatment groups after 3 days of treatment, while it cleared 5 days post-treatment with DA. Furthermore, relapse of infection occurred in the DA treated rats 13 days post-treatment; this occurred in the 50 and 100 mg/kg SEC combination groups 16 and 27 days post-treatment, respectively.

There was no relapse of infection in the 200, 400 and 800 mg/kg SEC combination groups up to day 70 post-treatment when the experiment terminated. It has been established that treatment of experimental *T. b. brucei* infection in animals becomes less effective as time interval between infection and treatment increases [20]. A possible explanation for this is the presence of trypanosomes in drug-inaccessible sites like the brain [21]. Treatment was initiated 8 days PI, which was 3 days post-confirmation of parasitemia. It appears that, the time interval between infection and treatment was long enough to allow trypanosomes to invade the brain, thus the relapse of infection. However, it appears that the combination of SEC at higher doses with DA lead to higher therapeutic drug concentration in the brain and other drug inaccessible sites, thereby preventing relapse of infection.

The effectiveness of SEC at higher doses could explain the high MIC observed in the *in vitro* assay. Furthermore, our findings suggest possible chemotherapeutic synergy between SEC and DA. However, the degree of effectiveness of this combination is dependent on the dose of SEC combined with 3.5 mg/kg DA. Lower doses of SEC combined with DA, though more effective than mono-therapy of DA, were not able to prevent relapse of infection. From this study, the lowest dose of SEC that can be combined with DA to prevent relapse of infection in rats was 200 mg/kg.

CONCLUSION

In vitro, SEC has been shown to possess anti-trypanosomal activity. *In vivo*, SEC exhibited a dose-dependent anti-trypanosomal effect. Combination therapy of SEC and DA prevented relapse of infection and was more efficacious than DA alone. The outcome of this study may

be very important in the treatment of trypanosomiasis in dogs, especially exotic breeds that reside in trypanosome-endemic areas which are very sensitive to DA.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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REFERENCES

1. *World Health Organization. Control and surveillance of African trypanosomiasis. A report of WHO expert committee. W.H.O technical report series 1998, no. 881.*
2. *Radostits OM, Blood DC, Gay CC. Veterinary Medicine. A text book of the diseases of cattle, sheep, pigs, goats and horses. 8th Ed. WB Saunders Co. Ltd. London, Philadelphia, Toronto, Sydney, Tokyo; 1994.*
3. *Thibault JM, Jaussaud P. Diminazene and its use in the dog. Note 1: Pharmacological and therapeutic aspects. Revue de Medecine Veterinaire (Toulouse, France) 1987; 138(1): 45-49.*
4. *Zimmer C, Wahnert U. Nonintercalating DNA-binding ligands: specificity of the interaction and their use as tools in biophysical, biochemical and biological investigations of the genetic material. Prog Biophysics Mol Biol 1986; 47(1): 31-112.*
5. *Peregrine AS, Mamman M. Pharmacology of diminazene: a review. Acta Trop 1993; 54(3-4): 185-203.*
6. *Onyeyili PA, Egwu GO. Chemotherapy of African trypanosomiasis: a historical perspective. Protozool Abstrs 1995; 19: 229–241.*
7. *Williamson J. Review of chemotherapy and chemotherapeutic agents. In: The African Trypanosomosis. (Edited by HW Mulligan and WH Pots). London, UK. George Allen and Unwin 1970; pp 125–221.*
8. *Murray PK, Jennings FW. African trypanosomosis. Chemotherapy in rodent models of sleeping sickness. In: Experimental bacterial and parasitic infections (Edited by Keush and Wadstrom). New York, USA, Elsevier Biochemicals, 1982; p 343.*
9. *Mariette D, Romaric N, Philippe T, Sylvie D, Pierrette C, Bruno B, Gerard C, Silla S, Philippe V.. Evaluation of trypanocidal drugs used for human African trypanosomosis against Trypanosoma lewisi. Parasite 2013; 20: 39.*
10. *Els T, Bernadette BT, David T, Marcel K, Reto B, Guy M, Michael AB, Bernard P. Fexinidazole – A New Oral Nitroimidazole Drug Candidate Entering Clinical Development for the Treatment of Sleeping Sickness. Plos Neglected Trop Dis 2010; 4: e923.*
11. *Onyeyili PA, Anika SM. Diminazene aceturate residues in the tissues of healthy, Trypanosoma congolense and Trypanosoma brucei brucei infected dogs. Brit Vet J 1991; 147: 155-162.*
12. *Nasiruddin AF, Anton AS, Sharma HK, Manavalan R. Analytical method development and validation of secnidazole tablets by RP-HPLC. J Pharm Res 2010; 2: 412-416.*
13. *Laurence L, Brunton LS, Goodman JSL. In Goodman and Gilman. The Pharmacological Basis of Therapeutics. McGraw-Hill Companies, Inc, 11th edition 2006.*
14. *Zhang LH, WU S. Pharmacological action and clinical application of secnidazole. (Institute of Materia Medica, Peking Union Medical College,Chinese Academy of Medical Sciences, Beijing 100050, China) Chinese New Drug J 2002; 10.*
15. *Rickman LR, Robson J. The blood incubation infectivity test: a simple test which may distinguish Trypanosoma brucei from T. rhodesiense. Bull Wld Hlth Org 1970; 42: 650–651.*
16. *Atawodi SE, Bulus T, Ibrahim S, Ameh DA., Nok AJ., Mamman M, Galadima M. In vitro trypanocidal effect of methanol extract of some Nigerian savannah plants. Afr J Biotechnol 2003; 32: 317-321.*
17. *Eke IG, Ihedioha TE, Ejimofor FK, Eze IO, Ezeudu TA, Eze UU, Okoye CN, Anaga AO. Evaluation of the Methanol Root Extract of Securidaca longipedunculata for Antitrypanosomal Activity in vitro and in vivo. Thai J Pharm Sci 2015; 39: 155-159.*
18. *Herbert WJ, Lumsden WH. Trypanosome brucei: "a rapid matching method" for estimating the host's parasitemia. Exp Parasitol., 1976; 40: 427-431.*
19. *Allam LD, Ogwu D, Agbede RIS, Sackey AKB. Hematological and serum biochemical changes in gilts experimentally infected with Trypanosoma brucei. Vet. Arhiv 2011; 81: 597-609.*
20. *Jennings FW, Whitelaw DD, Urquhart GM. The relationship between duration of infection with T. brucei in mice and the efficacy of chemotherapy. Parasitol 1977a; 75: 691–697.*

21. Jennings FW, Whitelaw DD, Holmes PH, Chizyuka HKB, Urquhart GM. The brain as a source of relapsing T. brucei infection in mice following chemotherapy. Int J Parasitol 1979; 9: 381–384.