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Effect of nitrobenzylthioinosinate on the toxicity of tubercidin and ethidium against *Trypanosoma gambiense*

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Summary

The coadministration of tubercidin and ethidium to mice infected with *Trypanosoma gambiense* gave a better parasite clearance than either of the single drugs. The combination was also more toxic to the mice but the inclusion of nitrobenzylthioinosinate in the therapy significantly alleviated the toxicity of the drug combination. Nitrobenzylthioinosinate per se had no trypanocidal activity and did not affect the trypanocidal action of the drugs. The biochemical basis for the nitrobenzylthioinosinate action appears to be due to the reduction of access of the drugs to tissues or organs sensitive to the toxic drugs. The potential for the use of this compound with nucleoside analogue compounds in the therapy of African trypanosomiasis is suggested.

Key words: *Trypanosoma b. gambiense;* ethidium; tubercidin; nitrobenzylthioinosinate; drug toxicity.

Introduction

Resistance to trypanocides in current use have created problems in the control and eradication of trypanosomiasis in Africa (Gill, 1971; Gray and Roberts, 1971). A rational use of combination therapy as a means of slowing or inhibiting the development of resistance or as a means of attacking resistance has not been well studied. Some studies on combination therapy such as the suramin-tryparsamide complex; isometamidium-dextran complex and salicylhydroxamic acid-glycerol combination have given encouraging results (Williamson, 1957; Stephen, 1958; Borst, 1977; Brohn and Clarkson, 1978; Aliu and Chineme, 1980).

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Table 1. Determination of LD50 for tubercidin and ethidium in mice

Agent dosage (mg/kg)	Number of mice alive on day 7	LD50
Tubercidin		
12.5	4/10	
10.0	5/10	
8.0	7/10	10.7
6.3	8/10	(7.5–13.9)
4.0	9/10	
3.2	10/10	
Ethidium		
120	0/10	
100	5/10	
75	7/10	74.1
50	8/10	(48.3–99.8)
25	10/10	
Tubercidin-ethidium		
10.0 / 12.5	1/10	
5.0 / 12.5	3/10	
Tubercidin-ethidium-NBMPR-P		
10.0 / 12.5 / 5	10/10	
5.0 / 12.5 / 5	10/10	

Male BALB/C mice were given single i.p. injections of tubercidin, ethidium and nitrobenzylthioinosinate either singly or in combinations in dosages shown in the table. Each mouse received an injection volume of 0.2 ml per 20 g body weight whether one or more drugs were given. Deaths were recorded daily for 7 days.

Tubercidin and ethidium as single agents have been demonstrated to possess trypanocidal activity (Hawking, 1963; Williamson, 1969; Williamson and Scott-Finnigan, 1975). Tubercidin is toxic to animals but its toxicity to experimental animals have been reduced when given in combination with nitrobenzylthioinosine (NBMPR) (Paterson et al., 1979a). NBMPR and its 5′-monophosphate derivative, nitrobenzylthioinosinate (NBMPR-P) are S^6-derivatives of 6-thioinosine and they are specific inhibitors of nucleoside transport in various animal cells (Cass and Paterson, 1977; Lynch et al., 1978). NBMPR binds tightly but reversibly to specific membrane sites on various animal cells: HeLa cells (Lauzon and Paterson, 1977); erythrocytes (Pickard et al., 1973); fibroblast cells (Eilam and Cabantchik, 1977). NBMPR protected cells in culture against toxic concentrations of tubercidin (Warnick et al., 1972; Cass et al., 1975; Paterson et al., 1979a, 1979b).

The present study was undertaken to compare the trypanocidal effects of ethidium-tubercidin combinations with that of the single compounds and also to determine whether the coadministration of NBMPR-P would influence these effects.

Table 2. Inhibition of tubercidin toxicity in mice with nitrobenzylthioinosinate

Agent (dosage mg/kg)	Number of mice alive on the day 30
Tubercidin	
25.0	0/10
18.7	0/10
12.5	4/10
6.3	8/10
3.1	10/10
Tubercidin-NBMPR-P	
25.0	10/10
18.7	10/10
12.5	10/10
6.3	10/10
3.1	10/10

Groups of mice (10 per group) were given single doses of either tubercidin or tubercidin and NBMPR-P (5 mg/kg). The tubercidin were given at different doses as stated in the table. The mice were observed for 30 days during which deaths were recorded.

Materials and Methods

Animals. Male BALB/C mice (18–22 g) were obtained from the animal breeding unit of Nigerian Institute for Trypanosomiasis Research, Vom.

Trypanosomes: Trypanosoma brucei gambiense (T. b. gambiense) used in the experiment was an isolate from a male patient from Kwa district of Plateau State of Nigeria in 1968. It has been maintained by rat to rat passage and kept frozen in liquid nitrogen. It is a virulent strain, killing rats and mice in two to four days.

Chemicals. Ethidium bromide was from BDH Chemicals, Poole. Tubercidin was a gift from Dr. J. E. Grady, UpJohn Company, Kalamazoo, USA. Nitrobenzylthioinosinate was a gift from Professor A. R. P. Paterson, Cancer Research Unit (McEarchern Laboratory), University of Alberta, Edmonton, Canada.

Animal inoculation. T. b. gambiense was obtained from rats with parasitemia in the range of 5×10^7 to 1×10^8 parasites per ml of blood. The parasites were diluted with Tris buffer pH 7.2 to approximately 10^6 parasites per ml. 0.2 ml of the suspension was inoculated i.p. into male mice.

Acute toxicity tests. The acute toxicity of the drugs were assessed by giving single i.p. injections of ethidium or tubercidin to mice (10 mice per treatment group). The drugs were made in 0.15 M NaCl solution and the injection volumes were proportional to 0.2 ml per 20 g body weight. Deaths were recorded daily for a period up to 7 days.

In vivo tests. Groups of mice (5 mice per group) were given single doses of either tubercidin alone or tubercidin and NBMPR-P together. The untreated groups received saline only in which the compounds were made. Also, in another experiment, groups of mice showing approximately 10^7 parasites per ml of blood were given single doses of tubercidin, ethidium or their combinations with and without NBMPR-P. Daily deaths were recorded in both cases.

In vitro tests. The *T. b. gambiense* trypomastigotes were separated from the blood of infected rats by the method of Lanham and Godfrey (1970). The parasites were diluted with Ringer's phosphate buffered salts solution with 10 mM glucose supplemented with 2% calf serum; streptomycin (50 μg/ml) and penicillin (100 IU/ml) (Hawking, 1963). The parasites were treated or incubated with tubercidin alone or in combination with NBMPR-P. The control was incubated in the buffer without any additions. Samples from the incubation media were removed periodically for examination to determine the number of motile parasites.

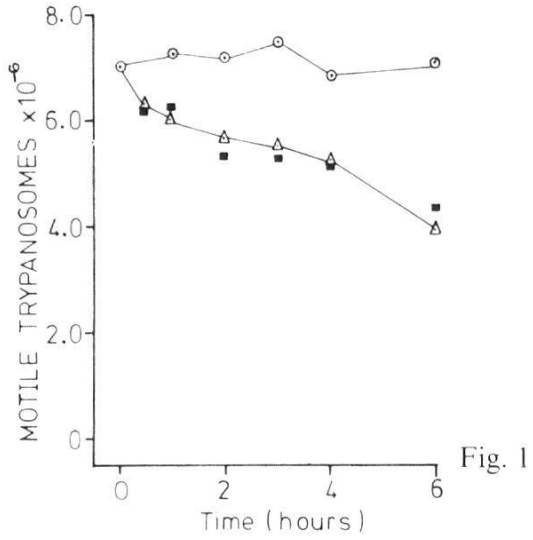

Fig. 1

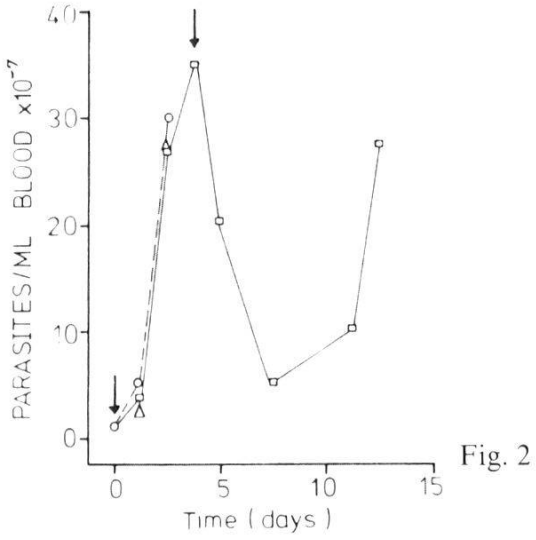

Fig. 2

Fig. 1. Effect of tubercidin on the motility of *T. gambiense* trypomastigotes in vitro at 26° C. *T. gambiense* trypomastigotes were incubated in the medium containing (a) buffer only to serve as the control (○); (b) 0.5 mM tubercidin (△); (c) 0.5 mM tubercidin and 5μM NBMPR (■). Samples were removed at intervals shown in the graph to determine the number of motile parasites.

Fig. 2. Trypanocidal activity of tubercidin with and without NBMPR-P. Group of mice inoculated with *T. gambiense* were treated with either normal saline (○) which served as the control; 5 mg/kg tubercidin (△) or tubercidin and NBMPR-P (□) at 5 mg/kg each. The drugs were given on the days indicated (↓). Blood samples from the tails of the mice were examined daily. All the mice in the control group and the group that received tubercidin alone died in 3–4 days.

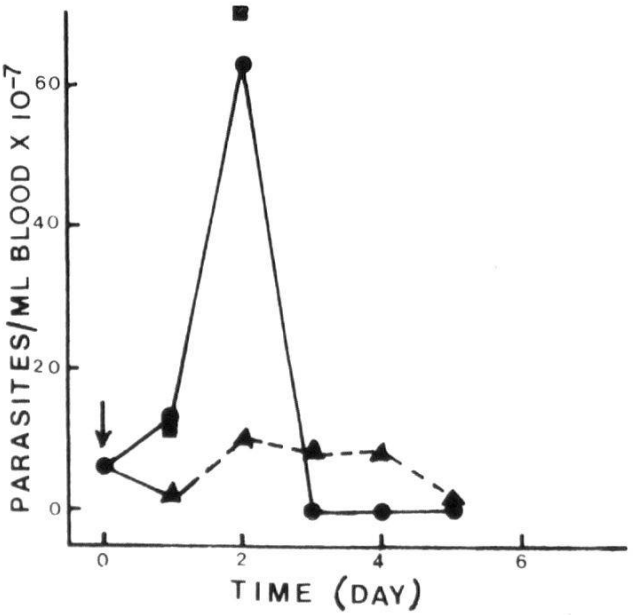

Fig. 3

Fig. 3. Efficacy of tubercidin and ethidium combination against *T. gambiense*. Groups of mice (5 mice per group) were inoculated with approximately 100,000 parasites and treatment began when parasitemia in the blood of the animal has averaged about 5×10^7 parasites per ml blood. Mice received single treatment (↓) as follows: (■) received tubercidin and NBMPR-P at 5 mg/kg each; (●) received ethidium (1 mg/kg) and (▲) received tubercidin (5 mg/kg), ethidium (1 mg/kg) and NBMPR-P (5 mg/kg). The blood from the tails of the mice was examined daily for parasites for 7 days. The plot shows average number of parasites per ml blood from mice in each the treatment group. Two mice in the group that received ethidium-tubercidin combination was completely cleared of parasites and they survived beyond 30 days observation period.

Results

It is apparent from the result summarized in Table 2 that NBMPR-P significantly alleviated the toxicity of tubercidin against mice. It was not possible to demonstrate a similar protection of mice against ethidium toxicity with NBMPR-P. The compound (NBMPR-P) per se was not toxic to mice at the tested dose (5 mg/kg). In the experiment shown in Fig. 2, the concurrent administration of tubercidin and NBMPR-P suppressed parasites growth in the blood of infected mice. The NBMPR-P did not block the trypanocidal action of tubercidin. This later finding, that is the non interference in trypanocidal action of tubercidin by NBMPR-P, is clearly illustrated in the in vitro experiment (Fig. 1) where NBMPR and tubercidin together in the incubation medium had a similar effect on the motility of the *T. gambiense* trypomastigotes as the tubercidin alone. A single treatment of infected mice with either 1 mg/kg ethidium or 5 mg/kg tubercidin was found to clear the *T. gambiense* parasites from the blood of the infected mice, but the combination of both drugs proved more effective as seen in Fig. 3. The increased toxicity of the combined drugs was alleviated by coadministration of the drugs with NBMPR-P (Table 1).

Discussion

The toxicity of nucleoside drugs against mice have been shown to be reduced or inhibited when NBMPR-P was given concurrently with the nucleoside analogue drugs (Paterson et al., 1979a). It is also demonstrated in the present study that NBMPR-P given along with tubercidin significantly inhibited the toxic effects of the drug against mice. NBMPR binds to a variety of animal cells; the binding to the cells correlated with the inhibition of uptake of nucleosides by the cells (Cass et al., 1974; Eilam and Cabantchik, 1977; Lauzon and Paterson, 1977). It would thus appear that the protection afforded by NBMPR-P to mice against tubercidin toxicity was as a result of the blockage of uptake of the toxic drugs by the animal cells. NBMPR-P did not affect the trypanocidal action of tubercidin. This observation correlated with the lack of NBMPR binding sites on *T. gambiense* trypomastigotes (Ogbunude and Ikediobi, 1982). The nucleoside transport inhibitor which protected the host cells against tubercidin toxicity did not bind to the parasites and thus did not interfere with the uptake of tubercidin by the parasites.

The inability of NBMPR-P to protect mice against ethidium toxicity agrees with previously established finding that NBMPR is a specific inhibitor of nucleoside transport (Lynch et al., 1978; Cass and Paterson, 1979). The failure of 1 mg/kg ethidium to cure infected mice might indicate that either the strain of *T. gambiense* has grown resistant to the drug or that ethidium is not effective in the treatment of *T. gambiense* infection. Previous reports have shown that in general the phenanthridinium compounds are more active upon the members of *T. congolense* and *T. vivax* than upon *T. brucei* group (Hawking, 1963).

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