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The appearance of isometamidium resistant *Trypanosoma congolense* in West Africa

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Summary

The frequent reappearance of patent parasitemia, mainly *Trypanosoma congolense*, in cattle maintained under isometamidium prophylaxis in the Upper Volta indicated that drug-resistant forms might be appearing. To investigate this possibility, trypanosome stocks were isolated in mice, their isometamidium sensitivity estimated and compared to that of stocks isolated before drug use was widespread. Most *T. congolense* stocks isolated in 1982–1983 were 4–8 times less sensitive to isometamidium than those isolated in 1979–1980.

Key words: *Trypanosoma congolense;* cattle; isometamidium; drug-resistance.

Introduction

The use of trypanocidal drugs has enabled increased numbers of cattle to be kept in tsetse fly infected areas. At the present time the most widely used and readily available drugs are the phenanthridium derivative isometamidium (Trypamidium, Specia; Samorin, May and Baker Ltd) and diminazene aceturate (Berenil, Hoechst AG). Previous drugs are rarely used because of unacceptable levels of toxicity, tartar emetic, dimidium bromide, or increasing resistance in the field, quinopyramine salts (Antrycide Prosalt, Imperial Chemical Pharmaceutical Ltd), ethidium chloride (Novidium, Boots Pure Drug Co. Ltd) and pyrithidium bromide (Prothidium, Boots Pure Drug Co. Ltd); the latter two show cross-resistance with isometamidium (Williamson, 1970; Holmes and Scott, 1982).

Since 1982, in the region of Bobo-Dioulasso, Upper Volta, patent trypanosomiasis (mainly *Trypanosoma congolense*) was repeatedly found in cattle main-

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tained under isometamidium prophylaxis. At Samorogouan (70 km north-west of Bobo-Dioulasso) Zebu cattle treated with 0.5 mg/kg deep intramuscular injections every three months were losing condition and showing patent parasitemia. The dose was increased to 1 mg/kg which often lead to more severe local abscesses at the site of injection. Trypanosomes reappeared in the blood of some animals after only two weeks, and after six weeks many animals showed infection. It was not possible to say whether this was due to reappearance of the same trypanosomes or to reinfection by tsetse flies. Similar observations were made on cattle at the CRTA breeding station (Banankeledaga, 15 km north of Bobo-Dioulasso). However, the disease incidence was lower since there are no tsetse flies on the breeding station and only few in the surrounding pastures where the animals graze in the wet season. Again the majority of infections were *T. congolense.*

Bourn and Scott (1978) in Ethiopia and Küpper and Wolters (1983) in the Ivory Coast have reported similar findings. In these studies patent parasitemia, a few weeks after treatment, was taken as an indication of resistance but trypanosome stocks were not isolated. Formal proof and precise quantification of resistance requires the isolation of stocks.

To investigate the apparent field resistance in the Upper Volta, stocks of *T. congolense* were isolated from both Samorogouan and Banankeledaga cattle and their sensitivity to isometamidium assessed in mice. Fortunately, we had available trypanosome stocks which were isolated from Samorogouan and other areas near Bobo-Dioulasso in or earlier than 1980, when isometamidium was not so widely used. This enabled us to carry out a comparison of drug sensitivity. To examine how widespread the occurrence of resistant strains has become in this region the sensitivity of stocks from Karankasso (50 km, south-east of Bobo-Dioulasso, near the Koba River) and Samandeni (40 km, north of Bobo-Dioulasso, near the Black Volta River) were also investigated. These were obtained from cattle which were not themselves drug treated but were exposed to tsetse flies in areas located on the trekking route of drug-treated cattle.

The resistance of a trypanosome isolate can be measured in many ways and the result is usually expressed as the dose required to kill a suitable proportion of the organisms. In this study we chose to measure the minimum effective dose (MED) which was defined as that which clears trypanosomes from the blood of 4 mice for one or more days. MED is the dose which kills most individuals of a strain. This test has been used to compare three stocks of differing drug sensitivity to metamidium and the MED in mice was very close to the curative dose in cattle (Hawking, 1963).

Materials and Methods

Trypanosomes

All the trypanosomes used in this study were identified as *T. congolense* by morphology in Giemsa stained preparations and by movement in wet preparations of infected blood. Trypano-

somes Serengeti/71/STIB/212 were originally obtained from a lion in Serengeti, Tanzania, in 1971. Dr. W. I. Morrison (ILRAD, Nairobi, Kenya) kindly provided a cloned population (1180) derived from this stock. All other stocks were derived from infected cattle blood by passage in normal NMRI or BALB/c mice. Trypanosomes were cryopreserved in liquid nitrogen using glycerol as a cryoprotectant as soon as possible after isolation, usually after 2–3 passages in mice.

Parasitemia was assessed by microscopic ($\times 400$) examination of tail blood (Herbert and Lumsden, 1976); 40 fields were examined before a result of "undetectable" was recorded, i.e. $<\log_{10} 5.1$ organisms/ml blood. To calculate means of a group of mice, when necessary, undetectable parasitemias were assigned an arbitrary value of $\log_{10} 4.1$.

Isometamidium sensitivity assay

Cryopreserved trypanosomes were amplified in 2 NMRI mice and passaged into 20 normal 8–10 week old NMRI mice of either sex. When parasitemia exceeded $\log_{10} 7.5$/ml, mice were randomized into 5 groups of 4 mice each and weighed. Isometamidium (Trypamidium, Specia, Paris, France) was dissolved in double distilled water to a 1% w/v solution and dilutions made in phosphate buffered saline pH 7.2 to give 0.04–0.005% solutions. Groups of mice received 0.5, 1.0, 2.0 or 4.0 mg/kg intraperitoneally. One group was left untreated. Isometamidium solutions were always prepared the day of experiment. Parasitemias were followed daily for 6–7 days after drug inoculation. The minimum effective dose was taken as that which reduced parasitemia below the level of detection for one or more days in all 4 mice.

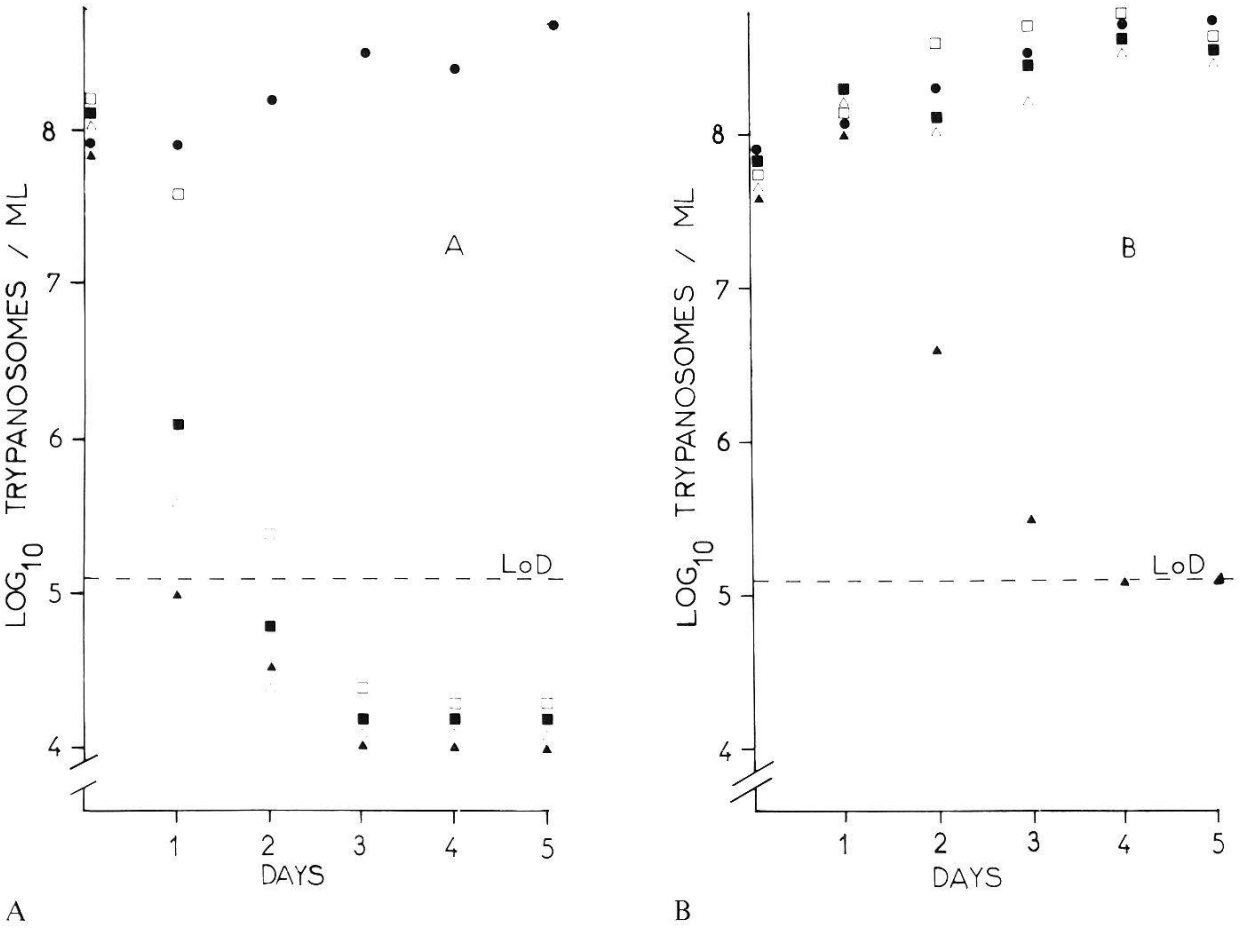

Fig. 1. Mean parasitemia in mice infected with Serengeti/71/STIB/212 (A) or Banankeledaga/83/CRTA/71 (B) following inoculation of isometamidium. Isometamidium was inoculated i.p. into groups of 4 mice each at dosages of □ = 0.5 mg/kg; ■ = 1.0 mg/kg; △ = 2.0 mg/kg and ▲ = 4.0 mg/kg. L.o.D. = limit of detection; ● = control no isometamidium.

Table 1. Sensitivity of *T. congolense* to isometamidium treatment in mice

Trypanosome[a]	Day parasitemia became undetectable isometamidium (mg/kg)[b]				Minimal effective dose (mg/kg)[c]
	0.5	1.0	2.0	4.0	
A. Serengeti/71/STIB/212	2.5	2.5	2.0	1.8	0.5
Farakoba/78/CRTA/19	3.0	3.3	3.7	3.0	0.5
Dinderesso/80/CRTA/3	3.0	2.3	2.0	1.3	0.5
B. Samorogouan/80/CRTA/20	P (2) 3.5 (2)	3.0	3.3	2.5	1.0
C. Samorogouan/82/CRTA/53	P	P	P (1) 3.0 (3)	2.7	4.0
Banankeledaga/83/CRTA/64	P	P	3.0	N.T.	2.0
Banankeledaga/83/CRTA/67	P	P	P	P (1) 3.0 (3)	>4.0
Banankeledaga/83/CRTA/68	P	P	P (2) 2 (3.5)	P (1) 3 (3.3)	>4.0
Banankeledaga/83/CRTA/71	P	P	P	3.3	4.0
Banankeledaga/83/CRTA/72	P	P	P (2) 3.0 (2)	4.3	4.0
D. Samandeni/83/CRTA/55	P	P	P (2) 2.5 (2)	3.0	4.0
Samandeni/83/CRTA/70	P (3) 3 (1)	P (2) 3.5 (2)	3.3	2.3	2.0
Karankasso/83/CRTA/61	P	P	P (1) 3 (3)	P (1) 2 (3)	2.0–4.0

[a] Serengeti is in Tanzania, all other isolates are from the Upper Volta.
[b] Arithmetic mean of day; P = parasitemia unaltered; figure in brackets refers to number of mice; N.T. = not tested.
[c] Dose at which all mice became aparasitemic.

Results

Treatment with isometamidium of mice infected with either of the 3 stocks Serengeti/71/STIB/212, Farakoba/78/CRTA/19 or Dinderesso/80/CRTA/3, resulted in rapidly falling parasitemia which became undetectable by around day 3, even for the lowest dose, 0.5 mg/kg, tested (Fig. 1A, Table 1A). Higher doses resulted in more rapid clearance of the Serengeti and Dinderesso stocks but that of the Farakoba stock was unaltered. A stock isolated from Samorogouan in 1980 (CRTA/20) was sensitive to doses of 1 mg/kg but 0.5 mg/kg was effective in only 2 out of 4 mice (Table 1B). Cure was not permanent in most instances and mice became parasitemic after a variable time lag.

Six stocks isolated in 1982 and 1983 (Samorogouan/82/CRTA/53 and Banankeledaga/83/CRTA/64, 67, 68, 71 and 72) were all resistant to 0.5 and 1.0 mg/kg. Four of these stocks were partially resistant to 2.0 mg/kg and two were partially resistant to 4.0 mg/kg (Fig. 1B, Table 1C). Three stocks were studied which were isolated from other areas near Bobo-Dioulasso (Table 1D). Two of these: Samandeni/83/CRTA/55 and Karankasso/83/CRTA/61 showed a similar pattern of resistance to the 1982–1983 Samorogouan and Banankeledaga stocks, the other showed partial resistance.

Discussion

Certain stocks of *T. congolense* isolated from the Bobo-Dioulasso region in 1979–1980 and one from E. Africa appear to be fully susceptible to isometamidium in the test used. The reappearance of parasitemia several days after treatment by this protocol in the mouse does not reflect resistance in the original isolate but is due essentially to rapid drug excretion following intraperitoneal or intravenous inoculation as opposed to the subcutaneous or intramuscular route (Hill and McFadzean, 1963).

Other stocks originating from cattle herds treated regularly with isometamidium show varying degrees of drug resistance. The drug resistance found in this study is not as pronounced as that reported after deliberate induction of resistance: Whiteside (1962) reported induction of trypanosome strains resistant to 40–80 times the curative dose. Whether the drug sensitivity found after mouse subinoculation quantitatively reflects that found in cattle needs to be investigated with these stocks. However, they were isolated from cattle treated regularly with 1 mg/kg isometamidium and the mouse effective dose was also greater than 1 mg/kg. The study of Hawking using metamidium (1963) indicates a close relationship between mouse effective dose and curative dose in cattle for three stocks. In his studies the mouse curative dose was at least 10 times higher than that required in cattle and this parameter is more difficult to estimate accurately with recent field isolates that often grow slowly in mice.

In the present comparative study it appears that most stocks isolated in 1982 or later are more resistant to isometamidium than earlier isolates. The stocks were isolated from different areas in western Upper Volta which indicates how widespread this phenomenon is becoming. These observations confirm that *T. congolense* is particularly prone to develop persistent drug resistant forms (Whiteside, 1962; Gray and Roberts, 1971; Leach and Roberts, 1981) and should be kept in mind when devising strategies for trypanocidal drugs (Williamson, 1970).

The sensitivity of the same stocks to diminazene aceturate is under investigation and some, but not all, show resistance (Authié and Pinder, submitted).

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