Drug resistance in recent isolates of Trypanosoma brucei and Trypanosoma congolense

R. A. Joshua

Introduction

Animal trypanosomiasis has nearly the same hold on the African continent today as it had about three decades ago (10). The provision of drugs for the treatment of the disease in animals has remained static in the last twenty-five years (17, 18) and hopes of new drugs coming into the market are very dim. The distribution of the disease therefore changes from time to time as efforts are made to control it. In the treatment of trypanosomiasis of cattle only isometamidium (Samorin™) and less toxic diminazene aceturate (Berenil™) have survived the waves of resistance development which followed the introduction of quinapyramine (Antrycide™) and homidium (Ethidium™) and led to their restricted use in large areas of Africa (7, 19).

Although chemoprophylaxis against bovine trypanosomiasis is widely practised in Africa, relatively few attempts have been made to assess its effectiveness. Available data indicate that the overall situation with regard to African trypanosomiasis in domestic livestock is deteriorating (9, 10). Current trypanosomiasis control measures which involve tsetse eradication or the use of trypanocidal drugs on domestic animals have been limited in their efficacy. Recent developments in the strategic use of drugs suggest that these approaches must be constantly evaluated with a view to improving the therapeutic efficacy in the control of trypanosomiasis in domestic livestock. A surveillance of drug resistance in recent isolates of trypanosomes gives a useful feedback in formulating chemoprophylactic control measures.

The present investigation was carried out to assess the drug susceptibility of recent isolates of trypanosomes obtained from domestic cattle in three different locations in the same ecological zone.

Materials and Methods

Animals and parasites

Blood samples were collected monthly during a two-year study period on herds of cattle kept at three different locations in the Jos Plateau i.e. Binchin, Ganawuri and Ryom.

At least 5 cc of heparinized blood was collected from each animal at each visit. Heparin was used at 10 I.U. per cc of bovine blood.

One Giemsa-stained thin film was made from each sample and the trypanosomes present were identified as described by HOARE (5). From each sample of cattle blood, 0.5 cc was inoculated into each of two mice. Parasitaemia in each mouse was monitored for twenty-five days.

Each infected mouse was bled from the orbital plexus as previously described by RILEY (13). Trypanosome-infected blood was cryo-preserved as previously described by CUNNINGHAM et al. (2). One monomorphic stock of each of T. brucei and T. congolense which has been maintained in the laboratory for over five
years was included as control for each species of trypanosome.

Drugs

BereniITM (diminazene aceturate, Hoechst Nig. Ltd.)
Two stock solutions were prepared. The first stock drug solution was 159 mg of drug granules dissolved in 100 cc of distilled water; 0.1 cc was injected intramuscularly per mouse. This corresponds to 3.5 mg/kg. The second stock solution was made by dissolving 159 mg in 50 cc of distilled water and 0.1 cc of the solution was injected intramuscularly into each mouse. This corresponds to 7 mg/kg body weight.

Stock drug solutions were stored at -20 °C when not used.

NovidiumTM (homidium chloride, Boots Nig. Ltd.):
Three stock solutions were prepared by dissolving 20 mg of drug in each of 100 cc, 50 cc, and 33 cc of distilled water respectively. Each mouse was given a single intramuscular injection of 0.1 cc of the appropriate drug dilution; 0.2 cc of the second solution was injected into each mouse for the 4 mg/kg dose.

SamorinTM (isometamidium chloride, May and Baker, Nigeria Ltd.): Two stock solutions were prepared by dissolving 10 mg of SamorinTM power in each of 100 cc and 50 cc of distilled water. Each mouse was given a single intramuscular injection of 0.1 cc of the appropriate drug dilution using a 1 cc hypodermic syringe and a 25 gauge needle.

Experimental animals

Balb/C mice that weighed between 18-20 g were used for trypanosome isolation and drug sensitivity tests.

Drug sensitivity test

Tests of drug sensitivity were carried out in batches of infected mice (generally 12).

Each sample of infected blood was retrieved from the cryobank and reconstituted into 3 ml of suspension in saline and inoculated intraperitoneally into each of twelve mice at 0.20 cc per mouse. Each group of infected twelve mice was divided into four subgroups of three mice each (A-D) and treated on day 5-8 post infection.

Subgroup A was treated with diminazene aceturate at 3.5 mg/kg.

Subgroup B was treated with homidium chloride at 1 mg/kg.

Subgroup C was treated with isometamidium chloride at 0.5 mg/kg.

Subgroup D was not treated with any drug but acted as controls.

All treatments for an isolate were carried out on the same day.

Parasitaemia in all of the mice was monitored for one month by microscopic examination of wet preparation from tail blood except in cases where death occurred before the expiration of the period. In the case of BereniITM and SamorinTM repeat tests were carried out using the double the initial dose. Further drug trials with homidium were carried out in double, treble and quadruple the normal therapeutic doses. In all cases repeat tests were carried out in freshly infected mice rather than relapsed infections. Tail blood from mice that failed to show trypanosoma as from the third day till thirty days post treatment were regarded carrying trypanocide susceptible parasites. On the other hand wet blood preparations from mice that indicated the presence of motile trypanosomes within the same period were regarded as showing resistant organisms.

A careful note was also taken of the period in days at which the motile organisms were first detected in the blood of all treated mice.

RESULTS

Infectivity of isolates to laboratory animals

During the investigations, eleven stocks of Trypanosoma congolense, five stocks of Trypanosoma brucei and fourteen stocks of Trypanosoma vivax were detected in thirty of the cattle sampled. The vivax organisms did not establish any persistent infections in mice. Microscopic examination of Giemsa-stained thin film showed that one cattle had a mixed infection of T. congolense and T. brucei.

The T. congolense and the T. brucei readily established infections in mice, hence the drug tests were carried out on them. However, no drug tests were carried out on the sample with a mixed infection.

Test of drug sensitivity were carried out in eleven isolates of T. congolense and five isolates of T. brucei.

Resistance to homidium chloride

Ten out of the eleven recently isolated stocks of T. congolense were resistant to normal therapeutic dose
TABLE I Isolates found resistant to normal therapeutic doses in various herds.

<table>
<thead>
<tr>
<th>Source of trypanosomes</th>
<th>Number isolated</th>
<th>Trypanosoma brucei</th>
<th>Trypanosoma congolense</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diminazene</td>
<td>Homidium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5 mg/kg</td>
<td>7 mg/kg</td>
</tr>
<tr>
<td>Binchin</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Ganawuri</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Ryom</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Laboratory stock</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

(1 mg/kg) of homidium chloride. When the dose was doubled five isolates were still resistant. Drug doses of 4 mg/kg however cured infections from nine isolates but two isolates were still resistant (Tables I, IV). All the brucei organisms were resistant to 3 mg/kg of homidium, increasing the drug dose to 4 mg/kg only resulted in a cure of one out of the five brucei isolates (Table III). The control T. brucei and T. congolense samples were readily susceptible to the drug at 1 mg/kg. It was also observed that the resistance was widespread in all the places sampled (Table II).

Susceptibility of diminazene aceturate

Drug dose of 3.5 mg/kg resulted in cure rate of five out of eleven isolates of T. congolense (Table I). Dose rate of 7 mg/kg resulted in a cure of ten out of eleven isolates. Thus one of the isolates was resistant to 7 mg/kg of the drug. The situation of T. brucei organisms are however different. All the isolates were resistant to diminazene aceturate at 3.5 mg/kg. However, only two were found resistant when 7 mg/kg drug was used.

Both control trypanosomes were readily susceptible to the drug at the recommended therapeutic dose.

Susceptibility to isometamidium

All isolates of T. brucei and T. congolense as well as control samples from the laboratory were readily susceptible to the drug at the recommended therapeutic dose (Tables I, II).

TABLE II Drug sensitivity of T. brucei and T. congolense isolates.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dosage</th>
<th>T. congolense</th>
<th>T. brucei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diminazene aceturate</td>
<td>3.5 mg/kg</td>
<td>5/11</td>
<td>0/5</td>
</tr>
<tr>
<td>Diminazene aceturate</td>
<td>7 mg/kg</td>
<td>10/11</td>
<td>3/5</td>
</tr>
<tr>
<td>Isometamidium chloride</td>
<td>0.5 mg/kg</td>
<td>11/11</td>
<td>5/5</td>
</tr>
<tr>
<td>Isometamidium chloride</td>
<td>1 mg/kg</td>
<td>11/11</td>
<td>5/5</td>
</tr>
<tr>
<td>Homidium chloride</td>
<td>1 mg/kg</td>
<td>1/11</td>
<td>0/5</td>
</tr>
<tr>
<td>Homidium chloride</td>
<td>2 mg/kg</td>
<td>6/11</td>
<td>0/5</td>
</tr>
<tr>
<td>Homidium chloride</td>
<td>3 mg/kg</td>
<td>9/11</td>
<td>0/5</td>
</tr>
<tr>
<td>Homidium chloride</td>
<td>4 mg/kg</td>
<td>9/11</td>
<td>1/5</td>
</tr>
</tbody>
</table>

* Recommended therapeutic doses of drugs for all trypanosomes.

TABLE III Effect of increased doses of homidium chloride on relapses by Trypanosoma brucei resistant to normal therapeutic doses.

<table>
<thead>
<tr>
<th>Drug doses</th>
<th>Number tested</th>
<th>Number resistant</th>
<th>Recrudescence of parasitaemia (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/kg</td>
<td>5</td>
<td>5</td>
<td>No remission</td>
</tr>
<tr>
<td>2 mg/kg</td>
<td>5</td>
<td>5</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>5</td>
<td>5</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>4 mg/kg</td>
<td>5</td>
<td>4</td>
<td>7.2 ± 2.3</td>
</tr>
</tbody>
</table>
Cross resistance

Infected mice were treated with only one drug. However, extrapolation of drug resistance results showed that some trypanosomes exhibited cross resistance. As shown in table V, one isolate of T. congolense and two isolates of T. brucei showed cross resistance to homidium chloride and diminazene aceturate. Such organisms were however readily susceptible to isometamidium.

Relapsed infection following drug treatment

As shown in tables III and IV increasing the dose of drug administered tended to lengthen the recrudescence of parasitaemia for both T. congolense and T. brucei in all treated mice with trypanocide resistant organisms.

<table>
<thead>
<tr>
<th>Drug doses</th>
<th>Number tested</th>
<th>Number resistant</th>
<th>Recrudescence of parasitaemia (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/kg</td>
<td>11</td>
<td>10</td>
<td>2.3 ± 0.7</td>
</tr>
<tr>
<td>2 mg/kg</td>
<td>11</td>
<td>5</td>
<td>6.4 ± 1.3</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>11</td>
<td>2</td>
<td>12.5 ± 2</td>
</tr>
<tr>
<td>4 mg/kg</td>
<td>11</td>
<td>2</td>
<td>15 ± 2.8</td>
</tr>
</tbody>
</table>

TABLE V Observed cross resistance in trypanosomes.

<table>
<thead>
<tr>
<th>Drug pairs</th>
<th>Number found resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homidium chloride and Diminazene aceturate</td>
<td>T. congolense 1  T. brucei 2</td>
</tr>
<tr>
<td>Homidium chloride and Isometamidium chloride</td>
<td>Nil  Nil</td>
</tr>
<tr>
<td>Diminazene aceturate and Isometamidium chloride</td>
<td>Nil  Nil</td>
</tr>
</tbody>
</table>

DISCUSSION

The present investigation has shown that one of the major problems encountered in the chemotherapy and chemoprophylaxis of animal trypanosomiasis is resistance to trypanocides. The very nature of disease control programme dictates that treatments must result in complete elimination of the targeted trypanosomes. Data from these tests agree with previously reported observation (6, 11, 14) that homidium resistant T. congolense are common in the field. In 1965 the Nigerian government’s ten-year long policy of using homidium as standard therapy was abandoned in favour of Berenil™ as a consequence of the discovery of homidium resistant T. congolense during a survey (11). The present study extended the earlier observation of widespread resistance to homidium and to a degree, to diminazene aceturate twenty years after the adoption of the drug. Until recently isometamidium has a restricted use in Nigeria. The observed drug resistance was found in trypanosomes from all the three locations studied. Earlier observations (11) demonstrated cross resistance to both isometamidium chloride and homidium chloride, however no cross resistance to isometamidium was demonstrated in the present study. The present study corroborates that of TOURATIER (16). Previous studies by GRAY and ROBERTS (4) showed that drug resistant trypanosomes are readily transmitted by tsetse flies and that transmissibility was found to be prolonged in one stock of T. congolense. Repeated fly passage was without influence on drug resistance. The latter observation might explain why homidium resistant trypanosomes still persist. The circumstances tending to encourage resistance to diminazene might be principally under dosing.

The present investigation did not reveal any cross resistance between diminazene and isometamidium and thus corroborates the earlier observation (3, 19) that diminazene and isometamidium are the preferred drugs for T. congolense infection in ruminants. The observed susceptibility to isometamidium by these trypanosomes should not be construed as a condemnation of other trypanocides but as an observation from a limited survey. LEWIS and THOMSON (8), PINDER and AUTHIE (12), AUTHIE et al. (1) observed resistance to isometamidium by T. congolense. The present results modify that of WILLIAMSON (19) that only isometamidium and diminazene have stood the wave of mass resistance by trypanosomes. It however confirms the observation of AUTHIE et al. (1) about resistance to diminazene aceturate and isometamidium in T. congolense isolates from Burkina.

The absence of proven and practical techniques of mass immunization underlines the continuing interest in the use of drugs for trypanosomiasis control.
Currently over 25 million trypanosomiasis treatments are given in Africa (15). The main constraints are cost and the need for suitable regimes to prevent the appearance of drug resistant trypanosomes. Regular surveillance of drug sensitivity of isolates will provide a useful pointer in the chemotherapy of trypanosomiasis. It is apparent from the present study that the alternate use of two drugs that do not cause mutual cross resistance might still provide reasonable prospect of control by chemotherapy.

ACKNOWLEDGEMENTS

Part of this work was carried out at the Nigerian Institute for Trypanosomiasis Research. I thank the Director for providing the facilities and Mr. Y. KAYIT for his technical assistance.


Studies were carried out in mice to assess the drug sensitivity of recent isolates of Trypanosoma brucei and Trypanosoma congolense. Each of eleven stocks of T. congolense and five stocks of T. brucei all isolated from cattle was tested for sensitivity to normal therapeutic dose of isometamidium chloride, diminazene aceturate and homidium chloride. Contemporaneous control tests were carried out on authenticated laboratory stocks of T. brucei and T. congolense. Six stocks of T. congolense were resistant to diminazene aceturate at 3.5 mg/kg but only one stock was found resistant to 7 mg/kg. Ten isolates of the T. congolense group were resistant to homidium chloride at 1 mg/kg. All the T. congolense isolates were susceptible to isometamidium chloride at 0.5 mg/kg. Two of the T. brucei were resistant to diminazene aceturate at 7 mg/kg while all were resistant to homidium chloride at even 3 mg/kg. All T. brucei isolates were sensitive to isometamidium chloride at 0.5 mg/kg. The control trypanosomes were readily sensitive to the three drugs at normal therapeutic doses. Key words : Trypanosoma brucei - Trypanosoma congolense - Drug resistance - Trypanocidal drug - Berenil - Novidium - Samorin.


Se utilizaron ratones para determinar la susceptibilidad a los medicamentos de Trypanosoma brucei y Trypanosoma congolense aislados recientemente. Se comprobaron cada una de las once cepas de T. congolense y de las cinco cepas de T. brucei aisladas a partir de bovinos para su susceptibilidad a la dosis normal terapéutica de cloruro de isometamidio, de aceturato de diminazeno y de cloruro de homidio. Simultaneamente, se efectuaron comprobaciones sobre cepas de laboratorio caracterizadas de T. brucei y T. congolense. Seis cepas de T. congolense eran resistentes al aceturato de diminazeno a la dosis de 3.5 mg/kg; una sola cepa fue resistente a 7 mg/kg. Diez aislamientos del grupo de T. congolense eran resistentes al cloruro de homidio a 1 mg/kg. Todos los T. congolense aislados eran susceptibles al cloruro de isometamidio a 0.5 mg/kg. Dos T. brucei eran resistentes al aceturato de diminazeno a 7 mg/kg mientras que todos resistían al cloruro de homidio, incluso a 3 mg/kg. Todos los T. brucei aislados eran susceptibles al cloruro de isometamidio a 0.5 mg/kg. Los tripanosomas testigos eran inmediatamente susceptibles a los tres medicamentos a las dosis terapéuticas normales. Palabras claves : Trypanosoma brucei - Trypanosoma congolense - Resistencia a los medicamentos - Tripanocida - Berenil - Novidium - Samorin.

REFERENCES


