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Selection for drug resistance in *Trypanosoma congolense* during cyclic transmissions through *Glossina morsitans morsitans* and drug treated rabbits

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Summary

A drug-sensitive *Trypanosoma congolense* (IL 1180 strain), with a known CD_{50} and CD_{90} (doses required to cure 50 and 90% of the infected animals) was cyclically passaged through tsetse flies. The infected flies were then fed on rabbits which received weekly prophylactic treatment of Samorin. It was observed that the infections arising from flies maintained for over 60 days on drug-treated rabbits required higher curative doses to achieve a 50 and 90% cure. The results of this work suggest that a selection for drug resistance occurs when trypanosome stage in *Glossina* is continuously exposed to drug-treated animals.

Key words: drug sensitivity; resistance; curative doses (CD_{50} and CD_{90}); *T. congolense;* Samorin.

Introduction

The extensive use of trypanocidal drugs has resulted in the development of drug-resistant strains of trypanosomes. The mechanisms by which the trypanosomes develop resistance to drugs are poorly understood, but the conditions that favour the development of drug resistance in the field have been reviewed by Leach and Roberts (1981). They pointed out that underdosing, the use of therapeutic and prophylactic drugs in areas of high trypanosome risk and the irregular treatment with prophylactic drugs are important factors enhancing the onset of drug resistance.

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Apart from the development of drug resistance, trypanocides of the phenanthridium group (e.g. Samorin and Ethidium) affect the normal functions of the kinetoplast, especially in the transformation process from the bloodstream to vector forms (Langley, 1975; Baker, 1977). The drugs cause a high percentage of dyskinetoplasty in trypanosome population (Newton, 1972; Vickerman and Preston, 1976; Riou et al., 1980), and they are likely to contribute as factors determining the trypanosome infection rate (Agu, 1984; and Nyeko et al., 1985).

We report here some findings on visible effects of Samorin (isometamidium chloride) on the morphology of trypanosomes in *Glossina* after ingesting blood from treated animals and the drug resistance arising from continuous feeding of the infected flies on animals treated with Samorin.

Materials and Methods

Trypanosome strain

Trypanosoma congolense (IL 1180 strain), a drug-sensitive strain, was obtained from the Kenya Trypanosomiasis Research Institute (KETRI), Muguga. The trypanosomes were cloned and the reserve stabilates prepared following the method described by Lumsden et al. (1973).

Tsetse flies

Glossina morsitans morsitans of both sexes, obtained from the International Centre of Insect Physiology and Ecology (ICIPE), were used in these experiments.

Trypanocide

Isometamidium chloride (Samorin) was purchased from the Veterinary Research Laboratories, Kabete. The appropriate weights of the drug required to obtain doses of 8 mg/kg body weight (bw) were weighed using Millibalance (model 75550 CAHN Electrobalance DTL) and by serial dilusions with distilled water, doses of 4, 2, 1, 0.5, 0.25 and 0.125 mg/kg bw were obtained.

Experimental animals

Randomly bred BALB C mice, weighing between 20–25 g, and New Zealand white rabbits, weighing 2 to 2.5 kg, obtained from the ICIPE animal house, were used.

Determination of CD_{50} and CD_{90}

Mice were inoculated with 0.1 ml of infected blood, containing 3×10^6 parasites ml. From day 2 post-inoculation, the mice were examined for infection by the standard parasitological techniques described by Murray et al. (1977). Infected mice with 1–20 parasites in the wet film per microscope fields (magnification × 400) were weighed, divided into groups of 5 and injected intraperitoneally with the appropriate volumes of freshly prepared Samorin solutions at doses stated above.

The control group received injections of saline. The mice were examined on days 1, 2, 3 and two times a week, for 30 days after treatment, to determine cure. Blood from mice that had no parasites by day 30 was passaged into clean mice and the mice examined for trypanosome infection for a further 30 days to confirm cure. The standard doses of Samorin required to cure 50 and 90% (CD_{50} and CD_{90}) of the treated mice were calculated using the probit regression analysis (Finney, 1952).

Exposure of infected tsetse to Samorin

Between 120 and 150 teneral flies were fed on infected mice at the first parasitaemic peak. The flies were maintained on clean untreated rabbits for 15 days after which they were divided into two groups. The first group of 80–100 flies was maintained for 30 days on rabbits that received a weekly prophylactic treatment of Samorin at 2 mg/kg bw. No flies were fed on rabbits within 6 h of

treatment. The other group of 40–50 flies was maintained for 30 days on rabbits that were injected with normal saline every week. After 30 days of continuous feeding on drug or saline treated rabbits both groups were fed on separate groups of clean mice in order to see if they would transmit trypanosome infection. The mice that became infected in this manner were used to infect another group of two sets of teneral flies. These sets of flies underwent the same experimental procedure as described above.

After the 2nd and 4th cyclic passages (giving a total of 60 and 120 days of continuous exposure of the infected flies to the drug or saline treated rabbits) drug sensitivity tests were performed to determine any changes in the CD_{50} and CD_{90}. The method described here for inducing drug resistance to the vector forms of trypanosomes is similar to the constant exposure diet-drug method described by Peters (1970) for malaria. Some flies were dissected within 24 h after ingesting drug-treated bloodmeal and the parasites were examined for activity or motility in gut or proboscis wet films and Giemsa-stained preparations examined for any aberrant trypanosomes.

Results

The doses of Samorin required to cure 50 and 90% (CD_{50} and CD_{90}) of mice infected with *T. congolense* IL 1180 strain were 0.15 ± 0.03 and 0.64 ± 0.103 mg/kg bw as shown in Table 1. These were taken as the standard curative doses before the start of cyclic transmissions.

Table 2 shows that maintaining infected flies on rabbits treated with Samorin resulted in a rise in the CD_{50} from 0.15 ± 0.03 to 0.46 ± 0.016 mg/kg bw and the CD_{90} from 0.64 ± 0.103 to 1.38 ± 0.026 mg/kg bw after four cyclic passages. As shown in Table 2, these increases in the CD_{50} and CD_{90} were significant ($P < 0.05$), no significant changes were observed in trypanosomes from the control group of flies.

The effects of Samorin on the morphology of the vector forms of trypanosomes in the Giemsa-stained preparations were swelling, vacuolisation, granulation and fragmentation. These changes were on the procyclic forms in the midgut and the metacyclic trypanosomes in the proboscis. Some trypanosomes were, however, perfectly normal.

Table 1. Number and percentage of mice cured following treatment with Samorin at different levels (*T. congolense* IL 1180 drug sensitive)

Samorin mg/kg bw	Mice cured per replicate (R)				
	R1	R2	R3	Total	% cured
4.0	5/5	5/5	5/5	15/15	100
2.0	4/4	5/5	5/5	14/14	100
1.0	5/5	4/5	5/5	14/15	93.3
0.5	3/5	5/5	5/5	13/15	86.6
0.25	3/5	3/5	4/5	10/15	71.4
0.125	2/5	3/5	2/5	7/15	46.6
Control					
0.0	0/5	0/5	0/5	0/15	0.0

Numerator denotes number of mice cured.
Denominator denotes number of mice treated.

Table 2. CD_{50} and CD_{90} of *T. congolense* (IL 1180 strain) after cyclic passages in the presence of drug – showing the mean CD ± SE, and the 95% confidence limits

Cyclic passages (CP)		Mean CD ± SE mg/kg bw	95% confidence limits		Significant* difference (Sd)
			lower	upper	
Before passages	CD_{50}	0.15 ± 0.03	0.07	0.23	
	CD_{90}	0.64 ± 0.103	0.524	0.746	
CP + Samorin for 60 days	CD_{50}	0.43 ± 0.014	0.40	0.46	sd
	CD_{90}	1.07 ± 0.02	1.025	1.115	sd
CP + Samorin for 120 days	CD_{50}	0.46 ± 0.016	0.426	0.494	sd
	CD_{90}	1.38 ± 0.026	1.354	1.406	sd
CP – No drug 60 days	CD_{50}	0.22 ± 0.022	0.198	0.242	No sd
	CD_{90}	0.80 ± 0.03	0.73	0.87	No sd
CP – No drug 120 days	CD_{50}	0.17 ± 0.026	0.11	0.23	No sd
	CD_{90}	0.67 ± 0.03	0.60	0.74	No sd

* The significant difference is in CD_{50} and CD_{90} after cyclic passages compared to those before passages.

No sd = $P > 0.05$
sd = $P < 0.05$

Discussion

By quantifying the CD_{50} and CD_{90} requirements of *T. congolense* (IL 1180 strain) before the start of our experiments, we are able to perform therapeutic experiments to determine if and how the levels of drug sensitivity changes during passages in the presence or absence of the drug. Conflicting results about the stability of drug resistance in trypanosomes during cyclical or syringe passages have been reported by earlier workers (Van Hoeve and Grainge, 1966; Willet, 1966; Gray and Roberts, 1971). These workers did not determine the actual curative doses of the trypanosome strains before the start of their experiments to compare with doses obtained following cyclical or syringe passages. This was necessary before any valid conclusions could be drawn.

Infected flies fed on animals regularly treated with Samorin did not lose their gut or proboscis infections. We, however, observed that a large number of trypanosomes in gut or proboscis wet films were immobile and dead. This was confirmed by the numerous granulation, fragmentation and swelling of such trypanosomes in the stained preparations.

Van Hoof et al. (1937) reported the attenuation of *T. brucei* group of trypanosomes and loss of the ability to transmit the infections by flies that were fed on animals treated with trypanocides. Hawking (1963) fed tsetse flies on bloodmeals containing various trypanocides and observed that most of the vector forms of trypanosomes were destroyed especially by Berenil and Samorin. Recently Agu (1984) observed a drastic reduction in the infection rate in

tsetse flies to *T. vivax* when he maintained the flies on bloodmeals containing Samorin. Our findings are in agreement with previous work, but we did not observe any loss of ability to transmit the infection by infected flies following treatment.

It was interesting to note that the infections arising from flies maintained for over 60 days on rabbits which received regular prophylactic treatment of Samorin required higher doses to achieve a 50 and 90% cure. Because of the frequent exposure to the drug in the tsetse, the trypanosomes, either by selection or induction acquired mechanisms of survival. This is shown by the relative resistance that developed following the continuous exposure of the infected flies to drug-treated bloodmeals. Indeed Riou (1976) and Jenni (personal communication) induced drug resistance to culture forms of trypanosomes by adding trypanocides into the media.

Leach and Roberts (1981) reported a number of factors which cause the development of drug resistance in the field. They pointed out, among other factors, underdosing, a high degree of trypanosomal challenge in conjunction with prophylactic treatment and the irregular application of prophylactic drugs.

Here we have described another factor, the long-term exposure of infected tsetse flies to the trypanocides leading to the development of drug resistance. In areas of high density and trypanosome challenge, the development of drug resistance as a result of constant prophylactic treatment is indeed a possibility to be borne in mind.

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