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The effect of trypanocidal drugs on the transmission of *Trypanosoma brucei brucei* by *Glossina morsitans centralis*

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Summary

The effects of trypanocidal drugs on *Trypanosoma b. brucei* infections in *Glossina m. centralis* have been investigated. Pentamidine and suramin exhibited no significant effects but both berenil and samorin reduced the number of salivary gland infections in comparison with controls. Berenil at concentrations of 10, 1.0 and 0.1 μg/ml significantly reduced the number of mature infections when fed to flies throughout the whole period of trypanosome development. A similar result was obtained with samorin at 0.1 μg/ml. Subsequent experiments showed that administration of both drugs at an early stage was more effective in preventing the maturation of infection than a later but more prolonged administration. Reported drug levels in the blood of different experimental host animals are of the same magnitude as those used here. It is suggested that repeated feeding of *T. b. brucei* infected *Glossina* on drug-treated hosts may reduce transmission, although alternative bloodmeal sources would reduce this effect. These influences are worthy of investigation in the field.

Key words: trypanocidal drugs; *Trypanosoma b. brucei;* vector stages; *Glossina m. centralis;* cyclical transmission.

Introduction

The efficacy of trypanocidal drugs is quite naturally assessed in terms of their action against bloodstream forms which occur in the host animal. However, the possible influence of therapeutic and prophylactic treatment of animals or man on trypanosome infections in the vector raises questions of importance in the epidemiology of trypanosomiasis. In a brief study Hawking (1963)

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showed that high levels of various trypanocidal drugs, including berenil and samorin (isometamidium), fed to *Glossina* in vitro appeared to eliminate most natural infections of *Trypanosoma vivax, T. congolense* and *T. rhodesiense.* Agu (1984) reported that maintenance of tsetse flies on samorin treated sheep eliminated both immature and mature infections of *T. vivax.* Conversely Nyeko et al. (1985) found that maintenance of *Glossina* on samorin treated animals did not affect transmission of *T. congolense* by the flies, but that the resulting infections showed an increased resistance to the drug. Thus it is possible that exposure of vector infection in the field to trypanocidal drugs will reduce transmission but that it may also lead to the appearance of more resistant forms of trypanosomes.

In this paper the effect of different drugs on the transmission of *T. b. brucei* by *G. m. centralis* is reported.

Materials and Methods

Trypanosomes: *Trypanosoma brucei brucei* STIB 247 is readily fly transmissible. It was isolated in 1971 in the Serengeti National Park from a hartebeest (*Acelaphus buselaphus*) and cryopreserved in liquid Nitrogen after one rat passage (Geigy and Kaufmann, 1973). After subsequent cyclical transmission the stock was cloned (247 I) and after 4 further mouse sub-passages restabilated as STIB 247 IA. STIB 247 IA or its derivatives were used to infect the flies used in these experiments.

Flies: *Glossina morsitans centralis* were obtained as pupae from ILRAD, Nairobi, Kenya. Prior to infection flies were cooled at 8 °C after emergence for up to 4 days. They were allowed to return to 27 °C before being offered an infective bloodmeal on mice at the peak of the first parasitaemia. Infected flies were then maintained at 26 °C and 70% relative humidity. Fresh pig blood was offered to flies through silicone membranes 3 times a week. In treated groups drug was added to the blood immediately before feeding. Preliminary experiments showed that it was necessary to mix the drugs with fresh blood. Freeze dried blood eliminated trypanocidal activity at low drug concentrations.

Metacyclic positive flies were identified by inducing flies to probe onto warmed glass slides. The infectivity of metacyclic forms was checked by feeding flies on white mice. All flies were subsequently dissected to determine infection rates and detect any late developing salivary gland infections not revealed by the probing technique.

Mice: White ICR male mice (25 g, Tierzuchtinstitut Zürich) were used to infect flies and to check for infective flies. For infection mice were inoculated intraperitoneally with stabilated trypanosomes, or with mouse blood obtained from previously infected mice. At the peak of the first parasitaemia mice were anaesthetized with nembutal (May & Baker, Essex), surface sterilized with alcohol and laid abdomen downwards on the cages of teneral flies to be infected. Fresh mice were used to check the infectivity of metacyclic and epimastigote positive flies. Each mouse was checked at least 5 times a week by the wet blood film technique for trypanosomes, for at least 30 days.

Drugs: these were obtained as marketed products from the following companies: Berenil – Hoechst AG, Frankfurt am Main, West Germany; Suramin (Germanin) – Bayer, Leverkusen, West Germany; Samorin (isometamidium) (Trypamidium) – Specia, Paris, France; Pentamidine (Lomidine) – Specia, Paris. All were in powder form except for pentamidine, which as Lomidine is marketed as a solution. All drug solutions were made up in distilled water at concentrations 100× greater than the final concentration required in the bloodmeal.

Results

Of the 4 drugs tested in these experiments: berenil, pentamidine, samorin and suramin, only 2 showed significant activity against trypanosomes in the fly

Table 1. Administration of different drugs in the bloodmeal to *Glossina m. centralis* infected with *T. b. brucei*

Drug	Dose μg/ml	Duration of treatment (days post-infection)	No. of treatments	Date of dissection (days post-infection)	Treated group: No. of flies examined	Treated group: No. of salivary gland infections (%)	Controls: No. of flies examined	Controls: No. of salivary gland infections (%)	Probabilities*
Suramin	50.0	2–18	8	20–29	47	13 (27.7)	30	13 (47.3)	N.S.
Pentamidine ..	1.0	3–21	8	24–25	61	13 (21.3)	65	16 (24.6)	N.S.
Samorin	0.1	2–18	8	21–26	73	0 (0.0)	60	25 (41.7)	<0.001
Samorin	0.1	2–7	3	28–29	44	9 (20.4)	48	16 (33.3)	N.S.
Samorin	1.0	2–7	3	28–29	53	5 (9.4)	48	16 (33.3)	<0.01
Samorin	1.0	2–7	3	21–23	44	0 (0.0)	43	11 (25.6)	<0.001
Berenil	1.0	2–19	7	23–31	34	0 (0.0)	63	17 (27.0)	<0.01
Berenil	1.0	3–19	8	23–28	63	1 (1.6)	64	21 (32.8)	<0.001
Berenil	0.1	2–21	9	23–26	56	15 (26.8)	41	22 (53.7)	<0.02
Berenil	1.0	8–19	6	23–28	70	13 (18.6)	64	21 (32.8)	N.S.
Berenil	1.0	2–7	3	23–26	48	10 (20.8)	41	22 (53.7)	<0.01
Berenil	10.0	2–7	3	21–23	50	5 (10.0)	43	11 (25.6)	<0.05

* statistical comparisons by chi-squared test; N.S. = not significant.

(Table 1). Pentamidine and suramin appeared to have no significant effect, although the number of mature infections in the treated groups was lower than in the controls. Both berenil (1.0 μg/ml), or samorin (0.1 μg/ml), however, almost completely eliminated mature infections from *Glossina* when offered repeatedly with the bloodmeal. In addition 0.1 μg/ml berenil significantly reduced the number of salivary gland infections in treated flies. Further experiments showed that when berenil was offered only at an early stage of the infection the number of salivary gland positive flies was still significantly reduced, whereas a delayed administration of the same dose more frequently had no apparent effect. Early stage administration of samorin also reduced the number of mature infections, although only at 1.0 μg/ml. The higher dose of berenil (10.0 μg/ml) was used to determine if midgut infections could be eliminated. However, as in all these experiments, there was no significant difference in the midgut infection rate as compared with the controls. Indeed, even the maturation of salivary gland infections was not completely prevented.

Discussion

The results presented here indicate that repeated uptake of the trypanocidal drugs berenil and samorin by *T. b. brucei* infected *Glossina m. centralis* reduces

the number of salivary gland infections. Even in experiments in which the number of mature infections was reduced to zero, however, the midgut infection rate was similar to that of the controls and proventricular infections were often found. This suggests that, while not killing trypanosomes in the midgut, these drugs prevent their transformation, probably to epimastigote forms which can invade the salivary glands and complete the cycle of development in the fly. The preferential interaction of berenil with kinetoplast DNA (kDNA) is well documented (Newton and Le Page, 1967; Newton, 1972) and samorin appears to have a similar mode of action (Jefferies, unpublished observations). It is known that kDNA contains many gene sequences only active in vector stages of the life cycle (Borst et al., 1985) and it is possible that the interaction of these 2 drugs with kDNA prevents the expression of genes important for transformation. The greater efficacy of berenil when administered at earlier stages of the infection appears to support this argument. If berenil is only added to the bloodmeal at a later stage (after day 8 P.I.), when proventricular infections are already present, the drug has no significant effect. Transformation has begun at this stage (Steiger, 1973), and forms in the proventriculus may be more resistant to drug action.

Hawking (1963) provided the first evidence that berenil and samorin could prevent the development of trypanosomes in tsetse flies. Drug doses used were, however, extremely high. Blood fed to flies contained 100.0 μg/ml blood, about 50 times the concentration in host blood after administration of samorin (Braide and Eghianruwa, 1980) and 50–100 times that of berenil (Kellner et al., 1985). However, the assessment of drug efficacy was based merely on the presence or absence of trypanosomes in dissected flies. The results presented here show that, in *T. b. brucei* infected flies, it is not necessary to eliminate midgut infections in order to prevent transmission and that drug levels present in host blood can achieve this end.

There is of course some danger in extrapolating the results obtained with an in vitro system to an in vivo situation, where the fly is feeding from a drug-treated host animal in which the drug concentration will be decreasing with time. However, drug levels in experimental animals in the literature are generally of the same magnitude, or greater than those used in the present experiments. Kellner et al. (1985) reported levels of berenil in the blood of calves treated with the standard dose of 3.5 mg/kg which exceeded 1 μg/ml for at least 5 days post-injection. After 20 days the concentration was still greater than 0.3 μg/ml. Concentrations of 0.2, 0.7 and 2.17 μg/ml of samorin have been reported in rats (Hill and McFadzean, 1963), camels (Ali and Hassan, 1984) and goats (Braide and Eghianruwa, 1980), respectively, 24 h after administration. Clearly then, it appears likely that *T. b. brucei* infected flies feeding repeatedly on berenil or samorin treated cattle may transmit the infection less frequently than would otherwise occur, although the availability of alternative bloodmeal sources would tend to reduce this effect. Such a situation may pertain on large,

well-managed ranches, e.g. at Mkwaja ranch, Tanzania, where chemoprophylaxis with samorin and berenil is practised on a regular and long-term basis (Trail et al., 1985). As *T. vivax* infections are also affected by samorin (Agu, 1984, 1985), an additional advantage of regular drug treatment may be a reduction in the rate of transmission of trypanosomiasis. However, it must also be conceded that frequent exposure of trypanosomes in the vector to drugs may lead to the occurrence of resistant populations, as reported by Nyeko et al. (1985), for *T. congolense* exposed to samorin during cyclical development in *Glossina.* At present, there is no evidence from the field to suggest that drug exposure in the fly reduces transmission or leads to drug resistance, but awareness of the possibility that this might occur when conducting field studies of trypanosomiasis could determine whether such effects have any importance in the epidemiology of the disease.

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