Effect of Isometamidium Chloride Treatment on Susceptibility of Tsetse Flies (Diptera: Glossinidae) to Trypanosome Infections

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J. Med. Entomol. 43(3): 564–567 (2006)

ABSTRACT Experiments were conducted to determine the effect of a single isometamidium chloride treatment of teneral tsetse flies, *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae), on the subsequent susceptibility to an infection with *Trypanosoma congolense* or *Trypanosoma brucei brucei*. Flies were offered a first bloodmeal on sterile gamma-irradiated defibrinated bovine blood that contained either 10 or 100 μg of isometamidium chloride/ml. Treated flies were subsequently infected with *T. congolense* IL 1180 or *T. b. brucei* AnTAR1 on day 3, 5, 10, or 20 posttreatment. To determine the effect of a single treatment with isometamidium chloride at 10 μg/ml on the fly's susceptibility to infection with isometamidium chloride-resistant trypanosome strains, treated flies were infected with one of two resistant isogenic *T. congolense* IL 1180 strains 3 d after the first feed. Results showed that a single isometamidium chloride treatment at 10 μg/ml blood sufficed to reduce significantly the fly's subsequent susceptibility to infection. Only 6.8% of the flies that were treated with isometamidium chloride developed a mature infection with *T. congolense* in the mouthparts compared with 34.3% of the control group. None of the flies that were administered isometamidium chloride and subsequently infected on day 3 or 6 with *T. b. brucei* developed a metacyclic infection in the salivary glands compared with 22.7% of the control flies. Likewise for the resistant *T. congolense* strains, a single treatment with isometamidium chloride significantly reduced the subsequent susceptibility to infection (6.5 and 33.5% of flies with metacyclic infections for treated and untreated flies, respectively). In practice and with respect to the release of sterile male flies to eradicate an isolated tsetse fly population, our results show that administering isometamidium chloride during the first bloodmeal (and before release) would significantly reduce the ability of these released males to transmit trypanosomes.

KEY WORDS *Glossina*, transmission, isometamidium chloride, *Trypanosoma congolense*, *Trypanosoma brucei brucei*

Tsetse-transmitted trypanosomiasis is one of the major constraints to sustainable rural development in a large part of sub-Saharan Africa. Recent work on the Island of Zanzibar has demonstrated that the release of sterile male tsetse flies can be an effective method to eradicate tsetse in isolated pockets and thus results in a permanent solution to the trypanosomiasis problem (Vreysen et al. 2000). However, a possible unwanted repercussion of the release of large numbers of sterile male flies is a significant increase in the number of potential vectors of trypanosomes in the initial phase of the control campaign. To prevent these sterile males from picking up trypanosome infections and acting as vectors, a first bloodmeal containing isometamidium chloride, a trypanocide with prophylactic action, is usually given before release. The effect of isometamidium chloride on trypanosome infections in the tsetse fly has already been documented in the literature. Hawking (1963) demonstrated that isometamidium chloride administered at a dose of 100 μg/ml blood was capable of clearing *Trypanosoma congolense*, *Trypanosoma vivax*, and *Trypanosoma brucei* infections in tsetse. Other workers, using tsetse maintained on sheep treated with isometamidium chloride (Agu 1984) or in an in vitro system of defibrinated cow blood containing 100 μg/ml blood (Agu 1985) showed that the drug was capable of eliminating mature and immature *T. vivax* infections in tsetse. Similarly, feeding infected *Glossina mortisans centralis* (Diptera: Glossinidae) on fresh pig blood containing isometamidium resulted in a significant reduction in the *T. congolense* and *T. brucei* infection rates (Jefferies and Jenni 1987a,b). However, recent findings show that a proportion of the tsetse population can still become infected with *T. congolense* trypanosomes after a first bloodmeal. Such infections could be prevented through prophylactic treatment of the released

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0022-2585/06/0564–0567$04.00/0 © 2006 Entomological Society of America

flies. The "prophylactic" effect of a trypanocide, administered to the first bloodmeal, on the ability of released flies to acquire a trypanosome infection has not been determined so far. Therefore, we performed a series of experiments to determine the efficacy of isometamidium chloride to prevent trypanosome development in the tsetse fly when administered to the fly's first bloodmeal. In the first series of experiments, we determined the prophylactic effect of two different doses of the drug. In the second series of experiments, the ability of the treated flies to transmit isometamidium chloride–resistant trypanosome strains was assessed.

Materials and Methods

Tsetse Flies. Male *Glossina morsitans morsitans* Westwood (<32 h old), from the tsetse fly colony at the Institute of Tropical Medicine of Antwerp (Belgium), were used in the experiments. The origin of this tsetse colony and the conditions of maintenance are described by Elsen et al. (1993).

Trypanosomes. *Trypanosoma brucei brucei* AnTAR1 (stock EATRO 1125) originally isolated from a bushbuck in Uganda (Van Meirvenne et al. 1975), and three isogenic clones of *T. congolense* IL 1180 were used in the experiments. The origin of these isogenic clones is described by Delespaux et al. (2005). These clones have different levels of resistance to isometamidium chloride and are genetically identical apart from the mutation(s) underlying the isometamidium chloride resistance phenotype. The susceptible clone (R_0) has a CD_{50} (the curative dose that gives complete cure in 50% of the animals) in mice of 0.018 mg/kg. The resistant clones have a CD_{50} of 1.8 mg/kg (R_{100}) and 3.6 mg/kg (R_{200}) for the low and high resistant clone, respectively.

Isometamidium Chloride Treatment of Flies. All trypanocide-treated flies received a first bloodmeal containing isometamidium chloride 32 h after eclosion (day 0). The drug was administered by feeding the experimental flies through a silicone membrane on defibrinated bovine blood that contained either 10 or 100 μg of isometamidium chloride (Samorin, Rhône-Mérieux, France)/ml blood. Sterile gamma-irradiated defibrinated bovine blood was obtained from the International Atomic Energy Agency (Vienna, Austria). To ensure that all experimental flies included in the experiment had ingested the trypanocide, only fully engorged flies were retained. After this first in vitro bloodmeal, all flies were fed on the ears of a healthy rabbit (in vivo), three times per week. The control fly groups were fed their first bloodmeal on isometamidium chloride-free defibrinated bovine blood.

Infection of Flies. Different groups of isometamidium chloride-treated and control flies ($n = 50$) received a single infective bloodmeal either on days 3, 5, 10, or 20 after their first bloodmeal. For all these infective bloodmeals, flies were fed on anesthetized NMRI mice that had an infection with either *T. congolense* or *T. b. brucei* at a parasitaemia of at least $10^{8.4}$ parasites per milliliter of blood estimated according to the scale of Herbert and Lumsden (1976). Only fully engorged flies were retained. To determine the effect of isometamidium chloride on the fly's infection rate with the resistant trypanosome strains, flies treated with 10 μg of isometamidium chloride/ml blood received an infective bloodmeal with the resistant strains 3 d after the first feed.

To avoid reinfection of the flies with *T. b. brucei* or *T. congolense* during the in vivo maintenance, rabbits were replaced at weekly intervals. Thirty days after the initial infective bloodmeal, all surviving flies were dissected using the method described by Lloyd and Johnson (1924). Their midgut and mouthparts or salivary glands were examined for the presence of trypanosomes.

Data Analysis. Statistical analyses were carried out in Stata 7 (StataCorp 2001). Two types of analyses were used. A Poisson regression was used to analyze the data from the experiments determining the effect of dose and age on the *T. congolense* and *T. b. brucei* infection rate. Considering the number or zero infections, preference was given to a Poisson regression over a logistic regression. Hereby, the flies' age, the isometamidium dose and the interaction between the

Table 1. Infection rates of male *G. m. morsitans* tsetse flies given a first bloodmeal on defibrinated bovine blood containing isometamidium chloride and subsequently infected with *T. congolense* (IL1180 clone R_0) on day 3, day 5, day 10, and day 20 after their first feed

Dose rate isometamidium chloride (μg/ml)	Day of infection	No. dissected	Proportion of infected flies		Maturation rate[a]
			Midgut (immature)	Mouthparts (mature)	
10	3	20	0.20 (4)	0.10 (2)	0.50
10	5	19	0.16 (3)	0.11 (2)	0.67
10	10	21	0.00 (0)	0.00 (0)	0.00
10	20	29	0.03 (1)	0.03 (1)	1.00
100	3	29	0.14 (4)	0.03 (1)	0.25
100	5	35	0.09 (3)	0.06 (2)	0.67
100	10	31	0.10 (3)	0.03 (1)	0.33
100	20	17	0.00 (0)	0.00 (0)	0.00
0	3	37	0.27 (10)	0.22 (8)	0.80
0	5	33	0.54 (18)	0.48 (16)	0.89
0	10	32	0.06 (2)	0.06 (2)	1.00
0	20	27	0.04 (1)	0.04 (1)	1.00

Flies were dissected 30 d postinfection.

[a] Maturation rate represents n mature infections/n midgut infections.

Table 2. Infection rates of male *G. m. morsitans* tsetse flies given a first bloodmeal on defibrinated bovine blood containing isometamidium chloride and subsequently infected with *T. brucei brucei* (AnTAR1 clone) on day 3, day 5, day 10, and day 20 after their first feed

Dose rate isometamidium chloride (μg/ml)	Day of infection	No. dissected	Proportion of infected flies		Maturation rate[a]
			Midgut (immature)	Salivary glands (mature)	
10	3	20	0.15 (3)	0.00 (0)	0.00
10	5	20	0.15 (3)	0.00 (0)	0.00
10	10	21	0.14 (3)	0.00 (0)	0.00
10	20	22	0.04(1)	0.00 (0)	0.00
100	3	33	0.06 (2)	0.00 (0)	0.00
100	5	32	0.00 (0)	0.00 (0)	0.00
100	10	27	0.18 (5)	0.00 (0)	0.00
100	20	22	0.04 (1)	0.00 (0)	0.00
0	3	32	0.22 (7)	0.16 (5)	0.71
0	5	34	0.44 (15)	0.29 (10)	0.67
0	10	28	0.18 (5)	0.07 (2)	0.40
0	20	29	0.07 (2)	0.00 (0)	0.00

Flies were dissected 30 d postinfection.
[a] Maturation rate represents *n* mature infections/*n* midgut infections.

two were used as explanatory variables. The number of flies dissected was entered as population exposed. A Poission regression is appropriate for binomial data provided that the proportion of infection is low and that the population exposed is taken into account. The comparison of the infection rates of flies infected with resistant *T. congolense* strains was carried out using a logistic regression.

Results

Feeding teneral tsetse flies a first bloodmeal that contained isometamidium chloride significantly reduced the fly's subsequent immature (midgut) and mature (mouthparts/*T. congolense* or salivary glands/*T. brucei*) infection rate compared with the control flies (Tables 1 and 2). This effect is visible in the experimental flies that received the infective bloodmeal within 5 d after the first bloodmeal. In these series, only 6.8% (7/103) of the flies that received isometamidium chloride in the first bloodmeal (regardless of the dose) finally developed a mature infection of *T. congolense* in the mouthparts compared with 34.3% (24/70) for the control group (Table 1). Moreover, none of the flies (0/105) that were administered isometamidium chloride and subsequently infected on day 3 or 6 with *T. b. brucei* developed a metacyclic infection in the salivary glands, whereas 22.7% (15/66) of the control flies were found positive upon dissection (Table 2). The levels of significance of the observed differences between the infection rates (*T. congolense* and *T. brucei*) of isometamidium chloride-treated flies (at 10 or 100 μg/ml blood) and the control flies are summarized in Table 3. The effects of isometamidium chloride on the mature *T. congolense* or *T. brucei* infection rate were not affected by the dose of isometamidium chloride ($P = 0.311$ and $P = 0.626$ for *T. congolense* and *T. brucei*, respectively). In all fly groups (either treated with isometamidium chloride or untreated), the infection rates decreased significantly when flies were infected on day 10 or 20 postemergence.

A single treatment with isometamidium chloride (10 μg/ml blood) also significantly ($P < 0.001$) reduced the fly's subsequent immature (or midgut) and mature (proboscis) infection rates when infected with *T. congolense* strains that are resistant to the drug (Table 4). In total, only 6.5% (13/201) of the flies that were administered isometamidium chloride and infected with one of the two *T. congolense* isometamidium chloride-resistant strains developed a mature infection in the proboscis compared with 33.5% (65/196) for the untreated flies infected with the fully susceptible trypanosome strains

Discussion

These data show that a first bloodmeal on defibrinated bovine blood that contains isometamidium chloride significantly reduces the tsetse fly's ability to become infected with *T. congolense* or *T. brucei.* The effect of isometamidium chloride administration on the trypanosome infection rate can be observed for a period of at least 5 d after the meal containing the drug after which the tsetse fly (treated as well as untreated) susceptibility to a trypanosome infection declines substantially. For suppression of the *T. congolense* and *T. b. brucei* development in the fly, a dose of 10 μg of isometamidium chloride/ml blood sufficed to obtain this effect.

The underlying mechanism by which isometamidium chloride affects the fly's susceptibility for trypanosome infection is not clear. One possibility would be that the active compound resides in the fly for some

Table 3. Significance (*P* value) of the difference between the immature and mature *T. congolense* or *T. b. brucei* infection rates of isometamidium chloride-treated and control flies

Dose	Immature		Mature	
	T. congolense	*T. brucei*	*T. congolense*	*T. brucei*
10	0.038	0.07	0.016	0.029
100	0.004	0.002	0.001	0.004

Table 4. Infection rates of male *G. m. morsitans* tsetse flies given a first bloodmeal on defibrinated bovine blood containing 10 μ g/ml isometamidium chloride and subsequently infected on day 3 with one of two drug-resistant *T. congolense* strains (IL 1180 clones R_{100} or R_{200})

T. congolense strain[a]	Group	No. dissected	Proportion infected flies		Maturation rate[b]
			Midgut	Mouthparts	
R_{100}	Treated	104	0.11 (12)	0.07 (7)	0.58
R_{100}	Control	100	0.43 (43)	0.43 (43)	1
R_{200}	Treated	97	0.07 (7)	0.06 (6)	0.86
R_{200}	Control	96	0.24 (23)	0.23 (22)	0.96

[a] *T. congolense* IL 1180 R_{100} : CD_{50} = 1.8 mg/kg; and *T. congolense* IL 1180 R_{200}: CD_{50} = 3.6 mg/kg.
[b] Maturation rate represents *n* mature infections/*n* midgut infections.

period having a direct toxic effect on the trypanosomes in the midgut. However, the observed highly reduced infection rates in the treated flies of the *T. congolense* isogenic lines that are resistant to the drug directly conflicts with this possibility. Another possibility is that the isometamidium chloride administered during the fly's first bloodmeal affects the midgut microbial environment. It has been shown that isometamidium chloride has some bactericidal activity (Khafagi et al. 2003). Affecting this microbial fauna in the tsetse fly's midgut could result in less favorable conditions for the establishment and the growth of procyclic trypanosomes. This could offer an explanation for both the highly reduced establishment of immature infections as well as the reduced maturation rate that were observed in flies fed with isometamidium chloride. With respect to the latter, it has been shown previously that the maturation rate of established procyclic trypanosomes is positively correlated with their growth rate in the tsetse midgut (Dale et al. 1995). Hence, a detrimental effect on the growth rate of the procyclic trypanosomes will significantly reduce the maturation rate of these trypanosomes. Further studies of the effect of isometamidium chloride on the tsetse midgut environment would be valuable to give more insight into the factors that determine the vectorial ability of tsetse flies for trypanosomes.

Although past observations show an effect of the drug on established or establishing trypanosome infections, they substantially differ from our experimental setup where the "prophylactic" effect of a single bloodmeal containing isometamidium chloride has been evaluated. In practice and with respect to the release of sterile male flies to eradicate an isolated tsetse fly population, our results show that administering isometamidium chloride during the first bloodmeal (and before release) would significantly reduce the ability of these released males to transmit *T. congolense* or *T. brucei.*

Acknowledgments

The work presented in this article was supported by the International Atomic Energy Agency (research contract no. 12621).

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Received 20 October 2005; accepted 23 December 2005.