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Cross-Resistance Associated with Development of Resistance to Isometamidium in a Clone of *Trypanosoma congolense*†

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Resistance to isometamidium was increased 94-fold in a clone of *Trypanosoma congolense* (clone IL 1180) by repeated subcurative treatment of infected mice for 11 months. This was associated with 3.4-, 33-, and 4.2-fold increases in resistance to diminazene, homidium, and quinapyramine, respectively. Both *T. congolense* IL 1180 and the resistant derivative were able to undergo cyclical development in *Glossina morsitans centralis* tsetse flies, producing hypopharyngeal infection rates of 40.0 and 39.8%, respectively.

In sub-Saharan Africa, treatment and prophylaxis of trypanosomiasis in cattle, sheep, and goats is dependent on the use of three compounds: diminazene, an aromatic diamidine; homidium, a phenanthridine; and isometamidium, a phenanthridine-aromatic amidine (8). Quinapyramine, a quinoline pyrimidine, is recommended for use against trypanosomiasis only in camels and horses (8, 13). All four compounds have been used in the field for more than 30 years, and resistance to each compound has been reported in trypanosome populations in a number of countries across Africa (14). Furthermore, in some instances, multiple drug resistance has been reported (2, 3, 9, 16) and is a particular threat to livestock production. The origin of multiple resistance is unclear but may be associated with cross-resistance, due to the closely related chemical structures of the different compounds. From work in the field, Whiteside (21) suggested the cross-resistance phenotypes that may occur as a result of the development of resistance to diminazene, homidium, isometamidium, or quinapyramine. However, such conclusions were not confirmed by controlled laboratory studies.

Among the antitrypanosomal compounds mentioned above, isometamidium (1) is the drug most commonly used chemoprophylactically for bovine trypanosomiasis (7). In this report we describe the cross-resistance phenotype associated with the development of resistance to isometamidium in a clone of *Trypanosoma congolense*, an economically important pathogen of domestic livestock in sub-Saharan Africa.

The study used *T. congolense* IL 1180, a doubly cloned derivative of an isolate collected in the Serengeti, Tanzania (5, 12). The isometamidium chloride 50% curative dose (CD_{50}) for this clone in mice is 0.018 mg/kg of body weight (b.w.). Thus, by comparison to other trypanosome populations, the clone is highly sensitive to isometamidium (15).

After infection of nonirradiated outbred Swiss white mice with *T. congolense* IL 1180, repeated subcurative treatment with isometamidium chloride (Samorin; RMB Animal Health Ltd., Dagenham, United Kingdom), as described previously for the induction of resistance to quinapyramine (13), was used over an 11-month period to progressively increase the resistance of the trypanosome population to this compound. After 5, 7, and 11 months, trypanosome aliquots were stored in liquid nitrogen (4) and were designated *T. congolense* IL 3341, *T. congolense* IL 3342, and *T. congolense* IL 3343, respectively. By the aforementioned protocol, *T. congolense* IL 1180 was passaged 4, 5, and 8 times, respectively, to produce these populations.

In order to evaluate the susceptibilities of *T. congolense* IL 1180, IL 3341, IL 3342, and IL 3343 to isometamidium chloride (Samorin), diminazene aceturate (Berenil; Hoechst, Frankfurt, Germany), homidium chloride (Novidium; RMB Animal Health Ltd.), and quinapyramine sulfate (Trypacide; RMB Animal Health Ltd.) in mice, the methodology of Peregrine et al. (15) was used. In brief, groups of mice were infected with 10^6 trypanosomes and were treated 6 h later with various doses of each antitrypanosomal compound. Thereafter, the animals were monitored three times a week for 60 days for the presence of parasites in wet films of tail blood. The drug susceptibilities of each trypanosome population were then expressed as a CD_{50} and were compared by standard logit analyses.

Over the 11-month period of drug selection, the resistance of *T. congolense* IL 1180 to isometamidium chloride was increased from a CD_{50} of 0.018 mg/kg of b.w. to a CD_{50} of 1.7 mg/kg of b.w. ($P < 0.001$). When *T. congolense* IL 1180 and *T. congolense* IL 3343 were characterized for their susceptibilities to the other antitrypanosomal compounds, the diminazene aceturate CD_{50} was shown to have increased from 2.3 to 7.8 mg/kg of b.w. ($P < 0.05$), the homidium chloride CD_{50} increased from 0.37 to 12.1 mg/kg of b.w. ($P < 0.01$), and the quinapyramine sulfate CD_{50} increased from 0.23 to 0.97 mg/kg of b.w. ($P < 0.01$). The CD_{50}s for *T. congolense* IL 3341 and *T. congolense* IL 3342 for each of the antitrypanosomal compounds were intermediate between these two extremes (Table 1).

To determine whether the decreased susceptibility of *T. congolense* IL 3343 to isometamidium was associated with an alteration in the tsetse fly infectivity or the transmissibility of the population, the ability of *T. congolense* IL 1180 and *T. congolense* IL 3343 to undergo cyclical development was evaluated in teneral male *Glossina morsitans centralis* tsetse flies from the colony bred at the International Livestock Research Institute (ILRI). Individual East African × Galla goats, obtained from a trypanosomiasis-free area of Kenya, were infected with one of the two trypanosome populations. Seven days after the animals were found to be parasitemic by the buffy coat phase-contrast technique (11), batches of flies were

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TABLE 1. Susceptibilities of *T. congolense* populations to antitrypanosomal compounds in mice

Trypanosome population	CD_{50} (mg/kg of b.w. [95% confidence interval])[a]			
	Isometamidium chloride	Diminazene aceturate	Homidium chloride	Quinapyramine sulfate
IL 1180	0.018 (0.013–0.025)	2.3 (2.0–2.6)	0.37 (0.30–0.40)	0.23 (0.15–0.34)
IL 3341	ND	3.9 (3.6–4.2)	1.2 (0.9–1.6)	0.24 (0.15–0.38)
IL 3342	0.47 (0.44–0.49)	5.7 (5.4–6.1)	3.2 (2.1–4.8)	0.57 (0.48–0.68)
IL 3343	1.7 (1.4–2.0)***	7.8 (7.5–8.1)*	12.1 (5.8–25)**	0.97 (0.84–1.1)**

[a] ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$ (all P values are comparisons of IL 3343 with IL 1180); ND, not done.

fed on one occasion on individual goats. Thereafter, the flies were maintained on uninfected rabbits, and on days 4, 6, 7, 8, 10, 13, 16, 19, and 22 following the feeding on the infected goat, groups of 30 flies from each batch were dissected and examined for the presence of trypanosomes in the midgut, labrum, and hypopharynx. The remaining tsetse flies were dissected on day 25. Both populations produced similar infection rates in the midgut, labrum, and hypopharynx (Table 2). Furthermore, for both populations, labrum infections were first detected on day 6, while hypopharynx infections were first detected on day 8 (data not shown).

This work has shown that the level of resistance of the *T. congolense* IL 1180 clone to isometamidium was increased 94-fold over an 11-month period by repeatedly treating infected mice with subcurative doses of isometamidium chloride. On the basis of CD_{50}s in mice, the 94-fold increase in resistance was shown to be associated with a 3.4-fold increase in resistance to diminazene, a 33-fold increase in resistance to homidium, and a 4.2-fold increase in resistance to quinapyramine. Similar results were obtained with the parental clone, *T. congolense* IL 1180, and the most resistant derivative, *T. congolense* IL 3343, when their drug susceptibilities were characterized in vitro by using metacyclic trypanosomes (6). The high level of cross-resistance to homidium and the low level of cross-resistance to diminazene are consistent with the field observations of Stephen (19) and Whiteside (21) and confirm the rationale for using diminazene as a "sanative" combination with isometamidium in the field to abrogate the development of resistance to isometamidium (21). However, because of the slight, but significant, cross-resistance to diminazene that was observed in the work described here (Table 1), it is possible that long-term use of isometamidium, without the use of diminazene as part of a sanative pair, may be associated with the development of trypanosome populations that express resistance to both isometamidium and diminazene (14). In general, however, it appears more likely that concomitant resistance to isometamidium and diminazene is associated with the use of quinapyramine in cattle, sheep, or goats, since resistance to the compound appears to develop relatively easily in *T. congolense* and is usually associated with high levels of cross-resistance to isometamidium, diminazene, and homidium (13, 21).

TABLE 2. Infectivities of *T. congolense* populations for *G. morsitans centralis*

Animal no.	Trypanosome population	No. of flies dissected[a]	Infection rate (%) in:		
			Midgut	Labrum	Hypopharynx
CD 192	IL 1180	140	42.9	40.7	40.0
CD 193	IL 3343	128	43.8	39.8	39.8

[a] The flies (all male) were first fed on infected goats 7 days after trypanosomes were first detected. Data are a collective summation for dissections carried out 16, 19, 22, and 25 days later.

In recent work isometamidium has been shown to enter *T. congolense* bloodstream forms via a saturable transporter in the plasma membrane with a high affinity for the drug (22–24); once internalized the primary site of action of the compound appears to be the mitochondrial type II topoisomerase (17). Since homidium has been shown to compete for uptake of isometamidium in *T. congolense* bloodstream forms (20), and vice versa (14a), it appears that isometamidium and homidium are both internalized via the same transporter. Thus, because the V_{max} for uptake of isometamidium is decreased in drug-resistant *T. congolense* (10, 22), the high level of cross-resistance to homidium observed in the work reported here is likely to be associated with the same mechanism that is responsible for changes in the transport of isometamidium. Alternatively, alterations in an intracellular target shared by these drugs may be responsible for the parallel effects observed. Indeed, in the case of isometamidium, modulation of the mitochondrial electrical potential appears to be associated with resistance to the compound in *T. congolense* (22).

Finally, *T. congolense* IL 1180 and *T. congolense* IL 3343 were shown to produce similar infection rates and undergo similar rates of cyclical development in *G. morsitans centralis*. Thus, the development of resistance to isometamidium did not appear to affect these two epidemiologically important parameters in this trypanosome population. From studies on the growth rates and behaviors of *T. congolense* populations in goats, Sones et al. (18) suggested that isometamidium-resistant stocks are less viable than isometamidium-sensitive stocks. However, those investigators did not determine whether the same conclusion also applies to the behavior of trypanosome populations in tsetse flies. The work reported here has indicated that this may not be the case since, relative to *T. congolense* IL 1180, the decreased susceptibility of *T. congolense* IL 3343 to isometamidium did not appear to influence either the infection rate or the rate of cyclical development of the trypanosome population in *G. morsitans centralis*. However, since both trypanosome populations are laboratory derived, similar studies need to be carried out with wild-type populations of *T. congolense* that differ in their susceptibilities to isometamidium before any generalizations can be made from the data.

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