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Derivation and Characterization of a Quinapyramine-Resistant Clone of *Trypanosoma congolense*†

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Over a period of 208 days a quinapyramine-resistant population was derived in vivo from a quinapyramine-susceptible clone of *Trypanosoma congolense*: IL 1180. While the dose of quinapyramine sulfate required to cure 50% of mice infected with the parental clone was 0.23 mg/kg of body weight, the 50% curative dose for the resistant derivative, IL 1180/Stabilate 12, was greater than 9.6 mg/kg. This approximately 40-fold increase in resistance to quinapyramine was shown to be associated with an 8-fold increase in resistance to isometamidium, a 28-fold increase in resistance to homidium, and a 5.5-fold increase in resistance to diminazene. Cross-resistance to homidium and diminazene was also demonstrated in goats. Two clones derived from the drug-resistant derivative underwent cyclical development in *Glossina morsitans centralis*, producing mature infection rates of 39.6 and 23.9%. Thus, induction of resistance to quinapyramine in *T. congolense* IL 1180 was associated with cross-resistance to isometamidium, homidium, and diminazene and did not compromise the population's ability to undergo full cyclical development in tsetse flies.

One of the most important diseases reducing productivity of domestic livestock in sub-Saharan Africa is trypanosomiasis (3). The trypanosome species responsible for this disease complex in cattle, sheep, and goats include *Trypanosoma congolense*, *T. vivax*, and *T. brucei* (5). Methods to control trypanosomiasis include control of tsetse populations, the exploitation of trypanosome-tolerant livestock, and administration of antitrypanosomal drugs (16).

Chemotherapy of trypanosomiasis in domestic livestock is at present dependent upon the salts of a relatively small number of synthetic compounds: homidium, isometamidium, diminazene, and quinapyramine (Fig. 1). All four compounds have been on the market for at least 30 years, and there are now reports of drug resistance in *T. congolense* and *T. vivax* in many parts of Africa (14). Furthermore, because of the close chemical relationships between the compounds, the development of resistance to individual trypanocides often appears to be associated with cross-resistance to others (31, 33).

Between the 1950s and 1970s quinapyramine was widely used in Africa as a therapeutic and prophylactic agent in cattle (8, 26). Resistance to the compound appeared to develop rapidly (28, 34) and was often associated with concomitant cross-resistance to isometamidium, homidium, and diminazene (18, 31). However, it was impossible to ascertain whether the apparent cross-resistance actually was cross-resistance or reflected difficulties in interpreting field-derived data. In 1976, quinapyramine ceased to be manufactured (13) because of the ease with which resistance appeared to develop (8, 14, 22) and because of problems with drug toxicity (7). However, in 1984 the compound was reintroduced to the market, but only for use in camels (27). Despite the problems that appeared to occur when quinapyramine was previously used in cattle, the compound has recently been reintroduced in some African countries for use as a trypanocide in this livestock species. In light of the aforementioned concerns, the study described here was undertaken, under controlled laboratory conditions, to determine the ease with which resistance to quinapyramine could be induced in a clone of *T. congolense* and the cross-resistance phenotype of such a quinapyramine-resistant population.

The study used a doubly cloned derivative of a *T. congolense* Savannah-type isolate collected from a lion in the Serengeti, Tanzania: *T. congolense* IL 1180 (9, 15, 19). In mice, the isometamidium chloride and diminazene aceturate 50% curative doses (CD_{50}s) are 0.018 and 2.3 mg/kg of body weight (b.w.), respectively (25).

In an attempt to induce resistance to quinapyramine in IL 1180, 10 nonirradiated outbred Swiss white mice (group 1) were infected intraperitoneally with a stabilate of IL 1180 which had been suspended in phosphate-buffered saline–glucose, pH 8.0. Subsequently, the mice were monitored three times a week by examining wet-blood films of tail blood at ×250 magnification. When the level of parasitemia in one or more animals had attained 50 to 100 trypanosomes per field, all the mice were treated with quinapyramine sulfate (Trypacide sulphate; May and Baker Ltd., Dagenham, United Kingdom) at a dose of 0.005 mg/kg of b.w.; within 2 days the mice became aparasitemic. When trypanosomes reappeared and attained a level of parasitemia similar to that immediately prior to treatment, the same 10 mice were retreated with quinapyramine sulfate at double the dose. When the parasites subsequently reappeared following a period of aparasitemia, the animals were anesthetized and exsanguinated into sodium citrate (final concentration, 3% [wt/vol]). Blood from each animal was then pooled, and a volume of approximately 0.5 ml was frozen in liquid nitrogen after 10% (vol/vol) glycerol was added (6). The remainder of the trypanosomes were then used to infect a second group of 10 naive mice (group 2). A further 10 groups of mice were infected and treated in the same manner: every group was treated on two occasions with quinapyramine

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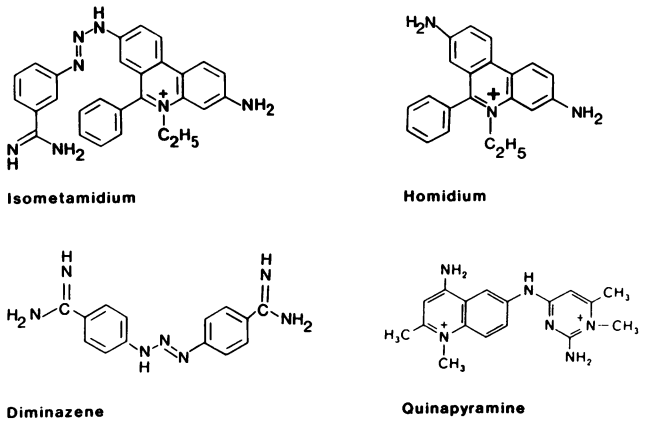

FIG. 1. Chemical structures of different trypanocides.

sulfate; the first dose was the same as that used for the second treatment of the previous group of mice; the second dose was greater than that used for the first treatment. Trypanosomes that reappeared in mice following the second treatment were used to infect the next group of naive mice. At each stage that a trypanosome population was passaged, a stabilate was prepared in liquid nitrogen. The final population that arose in group 12 was termed Stabilate 12.

Stabilate 12 was inoculated intraperitoneally into sublethally irradiated (650 rad) mice. Seven days later, when the animals were parasitemic, five clones were derived as described by Barry and Gathuo (2).

In order to characterize the drug sensitivities of IL 1180 and various derivatives in mice, stabilates of each population were expanded in sublethally irradiated (650 rad) mice and characterized as described by Peregrine et al. (25). Standard logit analyses were used to express the drug susceptibility of each population to each of the different trypanocides as a CD_{50}. The quinapyramine sulfate CD_{50} for the parental clone, IL 1180, was shown to be 0.23 mg/kg of b.w. (Table 1). In mice, the quinapyramine sulfate CD_{50} for IL 1180/Stabilate 12 was greater than 9.6 mg/kg of b.w.; a CD_{50} could not be determined since it was in excess of the maximum tolerated dose. To determine whether selection for resistance to quinapyramine resulted in cross-resistance to isometamidium (Samorin; May and Baker Ltd.), homidium (Novidium; May and Baker Ltd.), and diminazene (Berenil; Hoechst, Frankfurt, Germany), IL 1180 and IL 1180/Stabilate 12 were characterized for their susceptibilities to these three trypanocides in mice. The CD_{50}s for IL 1180 were 0.018, 0.37, and 2.3 mg/kg of b.w., respectively. In contrast, the same values for IL 1180/Stabilate 12 were 0.10, 10.35, and 12.74 mg/kg of b.w., respectively (Table 1). All five clones that were derived from IL 1180/Stabilate 12 had quinapyramine sulfate CD_{50}s in excess of 12.0 mg/kg of b.w. (Table 1; data for clones 2 to 5 not given), the maximum tolerated dose in these studies. Since clone 1 appeared to express a higher level of resistance than the other clones, on the basis of the rate of relapse following treatment, this clone was also characterized in mice for its susceptibility to isometamidium chloride, homidium chloride, and diminazene aceturate; CD_{50}s were 0.12, 6.83, and 8.34 mg/kg of b.w., respectively (Table 1). The clone therefore expressed a lower level of resistance to homidium and diminazene than did the population from which it was derived. However, the level of resistance to isometamidium was not significantly different.

In order to determine whether IL 1180/Stabilate 12/clone 1 expressed resistance to quinapyramine in a definitive host, the drug susceptibility of the population was also characterized in adult East African Maasai X Galla goats, maintained as described by Whitelaw et al. (30). Subsequent to expansion of IL 1180/Stabilate 12/clone 1 in sublethally irradiated (650 rad) mice, aliquots of 1.0×10^6 trypanosomes were inoculated intravenously into each of 22 goats (see below). The animals were then divided into four groups of five animals each (groups A1, B1, C1, and D1) and one group of two animals (group E1). Following inoculation of trypanosomes, jugular blood was collected from each animal three times a week and centrifuged, and the buffy coat was examined microscopically for the presence of trypanosomes (17). On day 3 following infection, when all animals were parasitemic, animals in groups A1, B1, C1, and D1 were treated with 3.0 mg of quinapyramine sulfate per kg of b.w., 0.25 mg of isometamidium chloride per kg of b.w., 1.0 mg of homidium chloride per kg of b.w., and 3.5 mg of diminazene aceturate per kg of b.w., respectively (the minimum recommended field doses for each compound). Animals in group E1 served as nontreated controls. In a second part to the experiment a further 10 goats were infected with IL 1180 in a manner identical to that used for animals in groups A1 to E1. After infection, the animals were divided into five groups of two animals each (groups A2, B2, C2, D2, and E2) and treated with the four aforementioned trypanocides as described above. Animals in group E2 served as nontreated controls. Following treatment, jugular blood was collected from all experimental animals three times a week for 150 days and examined for the presence of trypanosomes (17).

This experiment showed that IL 1180 was fully susceptible to each of the four drug doses in goats, with no trypanosomes detected in the treated infected goats. In contrast, infections with IL 1180/Stabilate 12/clone 1 relapsed in five of five goats after treatment with quinapyramine sulfate, in two of five goats after treatment with homidium chloride, and in three of five goats after treatment with diminazene aceturate. However, none of the five goats relapsed after treatment with isometamidium chloride. Thus, while IL 1180 was fully susceptible to the minimum recommended dose for each of the four trypanocides, IL 1180/Stabilate 12/clone 1 expressed resistance to quinapyramine sulfate, homidium chloride, and diminazene aceturate.

TABLE 1. Susceptibility of *T. congolense* IL 1180 populations to trypanocides in mice

Trypanosome	CD_{50} [mg/kg (95% confidence interval)] of:			
	Quinapyramine sulfate	Isometamidium chloride	Homidium chloride	Diminazene aceturate
IL 1180	0.23 (0.15–0.34)	0.018 (0.013–0.025)	0.37 (0.3–0.4)	2.3 (2.0–2.6)
IL 1180/Stabilate 12	>9.6	0.10 (0.09–0.1)	10.35 (9.7–10.9)	12.74 (11.7–13.7)
IL 1180/Stabilate 12/clone 1	>12	0.12 (0.11–0.13)	6.83 (6.5–7.1)	8.34 (7.6–9.0)

TABLE 2. Infectivity of *T. congolense* IL 1180 populations for *G. m. centralis*

Animal no.	*T. congolense* clone	No. of flies dissected[a]	Infection rate (%) in:		
			Midgut	Labrum	Hypopharynx
B 189	IL 1180	157	21.02	17.83	17.83
CJ 153	IL 1180/Stabilate 12/clone 1	169	56.80	41.42	39.64
CJ 154	IL 1180/Stabilate 12/clone 3	176	28.41	24.43	23.86

[a] The flies, all male, were first fed on animals 10 days after trypanosomes were first detected. Dissections were carried out 26 and 27 days later.

In a final experiment to determine whether induction of resistance to quinapyramine altered the ability of IL 1180 to undergo cyclical development, the tsetse transmissibility of IL 1180/Stabilate 12/clones 1 and 3 was ascertained in teneral male *Glossina morsitans centralis*, obtained from the International Laboratory for Research on Animal Diseases-bred colony, by standard procedures. Flies that extruded metacyclic trypanosomes in their salivary probes (4) were then fed on mice on a daily basis to determine the transmission rate of the trypanosome population. All the flies were then dissected to determine the infection rates. Both clones 1 and 3 were shown to undergo cyclical development, producing hypopharyngeal infection rates of 39.64 and 23.86%, respectively (Table 2). The transmission rates of the same clones were 100 and 90%, respectively. Thus, induction of resistance did not appear to affect the populations' ability to undergo full cyclical development in tsetse flies.

This study has therefore shown that the level of resistance of IL 1180 to quinapyramine was increased at least 40-fold by repeated subcurative treatment of infected mice over a period of 208 days. Furthermore, experiments to characterize the cross-resistance phenotype of the quinapyramine-resistant derivative in mice and goats indicated that induction of resistance to quinapyramine was associated with significant levels of cross-resistance to homidium, diminazene, and isometamidium.

While induction of resistance to quinapyramine in the clone of *T. congolense* described here was not difficult to achieve, attempts to induce resistance to quinapyramine in trypanosomes by other workers have met with various degrees of success. Using a similar procedure, Fiennes (8) failed to induce resistance in a stock of *T. congolense*. In contrast, a similar protocol successfully induced resistance to quinapyramine in *Trypanosoma equiperdum* (11, 24), *T. congolense* (12), and *Trypanosoma evansi* (10).

The cross-resistance phenotypes of trypanosome populations selected for resistance to quinapyramine, reported by different authors, have varied. A quinapyramine-resistant *T. equiperdum* population produced by Ormerod (24) expressed a high level of cross-resistance to dimidium and stilbamidine. Similarly, a quinapyramine-resistant stock of *T. congolense* generated by Hawking (12) was shown to be cross-resistant to diminazene. However, while a similar population of *T. evansi* was cross resistant to metamidium (a mixture of isomers, one of which was isometamidium [35]), the same population exhibited no cross-resistance to either stilbamidine or the organic arsenical tryparsamide (10). Finally, a population of *T. equiperdum* in which resistance to quinapyramine was induced appeared to be more susceptible to diminazene than the parental population (11). As a result of these data and other data concerning the drug resistance phenotypes of trypanosome populations isolated from field situations where quinapyramine was used, Whiteside (32) and Hawking (12) concluded that induction of resistance to quinapyramine leads to cross-resistance to phenanthridine compounds but does not always result in cross-resistance to diminazene, suggesting the induction of different mechanisms of resistance in different trypanosome populations. In the experiments described here, induction of resistance to quinapyramine in a cloned population of *T. congolense* resulted in significant cross-resistance to isometamidium, homidium, and diminazene. Since the salts of isometamidium, homidium, and diminazene are the only trypanocides currently marketed for use in cattle and the development of new trypanocides for use in cattle is now prohibitively expensive (33), the body of data generated to date would therefore indicate that use of quinapyramine in cattle as a trypanocide is contraindicated.

While there is a limited amount of information concerning the mechanism of action of quinapyramine in trypanosomes (1, 20, 21, 23, 29), we are unaware of data pertaining to the molecular basis for resistance to the compound. The trypanosome populations generated in this study represent ideal material for such work and are therefore currently being used in studies to determine the genetic and molecular bases of resistance to quinapyramine in *T. congolense*.

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