Expression of resistance to isometamidium and diminazene in *Trypanosoma congolense* in Boran cattle infected by *Glossina morsitans centralis*

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(Received 2 May 1989; accepted 28 June 1989)

Investigations were conducted on the sensitivity to isometamidium chloride (Samorin®) and diminazene aceturate (Berenil®) of derivatives of three of the *Trypanosoma congolense* stocks isolated between 1978 and 1983 from Zebu cattle in the Bobo-Dioulasso region of Burkina Faso. Boran cattle were used in the drug-sensitivity tests and were infected using *Glossina morsitans centralis*. The results showed that *T. congolense* stock IL 2466 isolated in 1978 was sensitive to the standard therapeutic dose of isometamidium chloride (0.25 mg kg⁻¹) and of diminazene aceturate (a.i. 3.5 mg kg⁻¹). However, *T. congolense* stock IL 2468 isolated in 1982 was resistant to both the prophylactic (0.5 and 1.0 mg kg⁻¹) as well as the therapeutic doses of isometamidium chloride (up to 1.0 mg kg⁻¹) although the sensitivity to the therapeutic dose of diminazene aceturate (3.5 mg kg⁻¹) was not affected. The *T. congolense* stock IL 2856 isolated in 1983 was highly resistant to the therapeutic action of diminazene aceturate (up to 10.5 mg kg⁻¹), as well as to the prophylactic (up to 1.0 mg kg⁻¹) and therapeutic action of isometamidium chloride (up to 2.0 mg kg⁻¹). The infection rates of the drug-resistant stocks of *T. congolense* in *G. m. centralis*, when goats were used as reservoir hosts, were as high (range, 22.3–56.3%) as of the drug-sensitive stock (49.5%). The resistance trait in the two stocks remained stable after their cyclical development in the tsetse vectors. The rate of transmission of the drug-resistant stocks to mice by the infected tsetse was also high (mean 81.3%).

**Key words**: Resistance; Isometamidium chloride; Diminazene aceturate; *Trypanosoma congolense*; *Glossina morsitans centralis*; Boran cattle

**Introduction**

Isometamidium chloride (Samorin®, May and Baker, U.K.) and diminazene aceturate (Berenil®, Hoechst, AG, F.R.G.) are the most widely used drugs against cattle trypanosomiasis in tsetse-infested areas of Africa. Whereas isometamidium chloride is used both as prophylactic and curative agent, diminazene aceturate is solely used as a curative drug (Williamson, 1970). These two drugs have been in use in the field as a sanative combination and they have been quite successful for the control of cattle trypanosomiasis in areas ranging from low to high trypanosome challenge (Blaser et al., 1977; Bourne and Scott, 1978; Wilson et al., 1981; Trail et al., 1985; Njogu et al., 1985). There is, however, evidence that isometamidium- or diminazene-resistant...
stocks of *Trypanosoma vivax* and *T. congo/ense* have developed in some countries (Whiteside, 1962; Bauer, 1962; MacLennan and Jones-Davies, 1967; Jones-Davies, 1967; Kupper and Wolters, 1983; Pinder and Authie, 1984; Röttcher and Schillinger, 1985; Schönfeld et al., 1987). Whereas cross-resistance to these two commonly used trypanocides in *T. vivax* and *T. congo/ense* has not been reported (Holmes and Scott, 1981), multiple drug resistant stocks of *T. vivax* have previously been described from Nigeria (Gray and Roberts, 1971a), Kenya (Röttcher and Schillinger, 1985; Schönfeld et al., 1987) and from Somalia (Schonefeld et al., 1987), and of *T. congo/ense* from Nigeria (Gray and Roberts, 1971a). Included amongst these drugs were isometamidium and diminazene aceturate.

Pinder and Authie (1984), and Authie (1984) isolated stocks of *T. congo/ense* from cattle in the Bobo Dioulasso region of Burkina Faso between 1978 and 1984. In their study, they determined the minimum effective dose (MED) of isometamidium chloride required to clear parasites from the blood of four mice for one or more days (Hawking, 1963), and found that most of the stocks isolated in 1982 or later were more resistant to the therapeutic dose of isometamidium chloride than those isolated earlier. Authie (1984) found that some of these *T. congo/ense* isolates were resistant also to different doses of diminazene aceturate. However, recent study has shown that although the result of a mouse test may give a broad indication of the sensitivity of a *T. congo/ense* stock to isometamidium chloride, it cannot be used to predict curative doses for cattle (Sones et al., 1988). These authors, however, did not determine the sensitivity of the stocks to isometamidium chloride when used for chemoprophylaxis.

In the present study, three of the *T. congo/ense* stocks isolated in Burkina Faso (Pinder and Authie, 1984) were investigated to determine their sensitivity to the prophylactic and therapeutic actions of isometamidium, as well as to the therapeutic action of the diminazene in Boran cattle which were infected using *Glossina morsitans centralis*.

**Materials and Methods**

**Trypanosomes**

Three of the stocks of *T. congo/ense* isolated from Zebu cattle in the Bobo-Dioulasso region of Burkina Faso were used (Pinder and Authie, 1984). The cattle in the region were maintained under isometamidium prophylaxis (0.5 to 1.0 mg kg⁻¹), the drug being given at three monthly intervals; whenever trypanosome infection was detected in cattle they were treated with diminazene aceturate (3.5 or 7.0 mg kg⁻¹) (Authie, 1984; Pinder and Authie, 1984). *T. congo/ense* IL 2466 was derived from the stock originally isolated from a cow at Farakoba in 1978 (Farakoba/78/CRTA/19). The primary stabilate was used to infect rats, then subpassaged three times in rats and cryopreserved as IL 2466. *T. congo/ense* IL 2468 was derived from the stock isolated from a bull at Samorogouan in 1982 (Samorogouan/82/CRTA/53). The primary stabilate was used to infect mice, then subpassaged four times in mice and cryopreserved as IL 2468. *T. congo/ense* IL 2856 was derived from the stock isolated from a bull at Banankeledaga in 1983 (Banankeledaga/83/CRTA/67). The primary stabilate was used to infect mice then subpassaged three times in mice, followed by four times in rats and cryopreserved as IL 2856.
Tsetse

Teneral male *Glossina morsitans centralis* were from the ILRAD-bred colony (Moloo et al., 1985).

**Animals**

Adult male castrated goats (crossbreeds between East African Maasai and Galla) weighing 20–25 kg were bought from an area of Kenya free from trypanosomiasis. Boran (*Bos indicus*) steers, 6 to 15 months of age at the start of the experiment were also obtained from an area of Kenya free from trypanosomiasis. They were screened for the presence of antibodies to *T. congolense*, *T. vivax* and *T. brucei* using an enzyme-linked immunosorbent assay (Voller et al., 1975) and an indirect immunofluorescent antibody test (Katende et al., 1987). Antibodies to these trypanosome species were not detected in any of the steers. All the animals were housed in maximum insect-proof isolation units, and allowed access to water and hay supplemented with concentrate ration.

Random-bred adult Swiss mice (FF1/OLA) used were from a colony bred at ILRAD. They were maintained on a commercial pelleted ration.

**Administration of isometamidium and diminazene aceturate**

Isometamidium chloride was administered by deep intramuscular injection into the middle third of one side of the neck. An 18 gauge, 3.5 cm needle was used and care was taken that none of the drug was deposited subcutaneously. Diminazene aceturate was administered by intramuscular injection into the thigh. Both drugs were administered to animals on the basis of an accurate bodyweight measurement taken immediately before treatment.

**Trypanosome infections**

Goats were used as reservoir hosts to infect tsetse. One ampoule of each of the three *T. congolense* stabilates was thawed at room temperature, diluted in 3 ml of phosphate-buffered saline-glucose (PSG), pH 8, and injected into the jugular vein. The infection was monitored daily, except on Sundays, by bleeding the animal from the ear vein and examination of the wet thin blood films for the presence of parasites by phase contrast microscopy at 400× magnification using a combination of Periplan 10× eyepieces and NPL Fluotar 40× objective (Leitz Wetzlar, F.R.G.). The packed red cell volume per cent (PCV) was also measured and the buffy coat was examined for the presence of parasites by the method of Woo (1969). Five days after parasitaemia was detected, 200 teneral tsetse were allowed to feed on the clipped flanks of each goat for 27 days. The tsetse were then left unfed for 2 days and on day 30, the surviving hungry tsetse were allowed to probe singly onto warmed slides at 37°C. To determine the mature infection rate, tsetse saliva was examined for the presence of metacyclic trypanosomes by phase contrast microscopy at 320× magnification using a combination of Periplan 10× eyepieces and a long-distance L32 objective. When the Boran steers were used as reservoir hosts to infect teneral tsetse, each animal was infected using five tsetse which had shown metacyclics in their salivary probes. Two hundred teneral tsetse were fed on the flanks on day 5 after
parasitaemia had been detected, and then maintained on a rabbit. Salivary probes were examined for the presence of metacyclics on day 30 after the infected feed.

To determine the transmission rate (TR), tsetse with mature trypanosome infections were fed singly on the abdomens of mice. The tail blood of the mice was subsequently examined three times a week for the presence of parasites by phase contrast microscopy at 400x magnification. Mice were monitored for infection for 40 days after the infected tsetse had fed on each animal.

Experiments and Results

*T. congolense* stock IL 2466

200 teneral tsetse were fed on a goat infected with *T. congolense* IL 2466. The infection rate in the tsetse on day 30 was 45.9%. The infected tsetse were then fed on the flanks of 4 Boran steers (Animals D-628, D-639, D-629 and D-644), 5 infected tsetse per animal. Full details are given in Fig. 1, and are summarised below:-

Fig. 1. Sensitivity of *T. congolense* stock IL 2466 (Farakoba/78/CRTA/19) to the therapeutic dose of isometamuseum chloride and diminazene aceturate in Boran cattle (numbers in the brackets indicate number of days; IR, infection rates in tsetse by probing on day 30; +ve and -ve, positive or negative for the trypanosome infections).
Animals D-628 and D-639: Infection was first detected on day 14 following the infected tsetse feeds, and both the animals were treated 9 days later with 0.25 mg kg\(^{-1}\) isometamidium chloride. In Boran D-628, parasitaemia was first detected on day 60 after treatment; thereafter parasites were detected intermittently during the observation period of 226 days. This animal was then administered 0.5 mg kg\(^{-1}\) isometamidium chloride and it remained aparasitaemic for up to 84 days when the experiment was terminated. However, Boran D-639 remained aparasitaemic for 193 days following treatment with 0.25 mg kg\(^{-1}\) isometamidium.

Animals D-629 and D-644: Infection was first detected on day 12 and 14, respectively, following the infected tsetse feeds. Both the animals were treated on day 11 and 14, respectively, after infection was detected, with 3.5 mg kg\(^{-1}\) diminazene aceturate. No parasites were detected by day 81 when the experiment was terminated.

T. congolense stock IL 2468

Therapeutic drug regime

200 teneral tsetse were fed on a goat infected with T. congolense IL 2468. The infection rate in the tsetse on day 30 by the warm-slide probe method was 56.3%. Two Boran steers, D-202 and D-203, were each infected using 5 infected tsetse. Full details of the experiment are given in Fig. 2 and are summarised below:

Animals D-202 and D-203: Infection was first detected on day 13 and the animals were treated 2 days later with 0.5 mg kg\(^{-1}\) isometamidium chloride. The infection relapsed in both animals on day 15 or 20 after treatment. They were then treated with 3.5 mg kg\(^{-1}\) diminazene aceturate and remained aparasitaemic for 204 days when the experiment was terminated. In the case of Boran D-203, 200 teneral tsetse were fed on it on day 5 after the infection had relapsed following isometamidium treatment. The tsetse were then maintained on a rabbit and probed on day 30; the infection rate was 22.6%. Two Boran steers, D-594 and D-608, were each infected with 5 of the infected tsetse.

Animals D-594 and D-608: Infection was detected on day 15 or 17 following the infected tsetse feeds on the two animals. Both animals were treated with 0.5 mg kg\(^{-1}\) isometamidium chloride. In Boran D-594, parasitaemia was detected on day 14 and the infection relapsed again after administration of 1.0 mg kg\(^{-1}\) isometamidium chloride. This animal was treated with 2.0 mg kg\(^{-1}\) isometamidium and the experiment terminated. Boran D-608 died of unknown cause 14 days after isometamidium administration and was aparasitaemic.

Prophylactic and therapeutic drug regimes

Teneral tsetse were fed on a goat infected with T. congolense stock IL 2974 which had been derived from IL 2468 in goat 2 (see Fig. 2). The infection rate in tsetse on day 30 was 46.1%. Six Boran steers were infected, each with 5 infected tsetse. Of these, 30 days prior to the tsetse-transmitted infection, 2 animals (E-188 and E-189) had been injected with 0.5 mg kg\(^{-1}\) isometamidium, 2 animals (E-177 and E-187) had been injected with 1.0 mg kg\(^{-1}\) isometamidium, whilst the remaining 2 (E-246 and E-247) served as the untreated controls. Full details of the experiment are given in Fig. 3 and are summarised below:

Animals E-188 and E-189: Infection was detected on day 15 following the infected tsetse feeds and both the animals were treated with 0.25 mg kg\(^{-1}\) isometamidium.
The infection relapsed. Moreover the infection in the two animals relapsed even after the further administration of isometamidium at 0.5 mg kg\(^{-1}\) and later at 1.0 mg kg\(^{-1}\). The two animals were then treated with 7.0 mg kg\(^{-1}\) diminazene aceturate; they remained aparasitaemic for upto 60 days when the experiment was terminated. In the experimental Borans E-177 and E-187 as well as in the control animals ED-246 and E-247, the relapse of infection, the drug administration and the results were similar to those for the above Borans E-188 and E-189.

**T. congolense** stock IL 2856

**Therapeutic drug regime**

200 teneral tsetse were fed on a goat infected with **T. congolense** IL 2856. The infection rate in tsetse probed on day 30 was 33.3%. Two Boran steers, D-204 and D-
Fig. 3. Sensitivity of *T. congolense* stock IL 2974 (Samorogouan/82/CRTA/53) to the prophylactic and therapeutic dose of isometamidium chloride as well as therapeutic dose of diminazene aceturate in Boran cattle. (For explanations of the abbreviations, see Figs. 1 and 2.)

500, were infected, each with five infected tsetse. Full details of the experiment are given in Fig. 4 and are summarised below:

**Animals D-204 and D-500:** Infection was detected on day 18 and the animals were administered 0.5 mg kg⁻¹ isometamidium. The infection relapsed in both the animals. They were treated with 3.5 mg kg⁻¹ diminazene aceturate but the infection relapsed at this drug dose and again at the higher dose of 7.0 mg kg⁻¹. They were then treated at the dose of 10.5 mg kg⁻¹ diminazene aceturate. Whereas animal D-204 remained aperasitaemic for 178 days of examination, in Boran D-500 the infection relapsed on day 52 following treatment. This experiment was terminated on day 178 following treatment. When the infection relapsed in animal D-500 following isometamidium treatment, 200 teneral tsetse were fed on it five days later and the tsetse were then maintained on a rabbit. On day 30 the infection rate in tsetse was 18.3%. Two Boran steers, D-617 and D-685, were infected with 5 infected tsetse per animal.

**Animals D-617 and D-685:** Infection was detected 18 and 17 days, respectively, following the infected tsetse feeds. Both the animals were administered 0.5 mg kg⁻¹ isometamidium but the infection relapsed at this drug dose and even at the higher dose of 1.0 and later at 2.0 mg kg⁻¹ isometamidium.
Fig. 4. Sensitivity of *T. congolense* stock IL 2856 (Banankeledaga/S3/CRTA/67) to the therapeutic dose of isometamidium chloride and diminazene aceturate in Boran cattle. (For explanations of the abbreviations, see Figs. 1 and 2.)

**Prophylactic and therapeutic drug regimes**

Teneral tsetse were fed on a goat infected with *T. congolense* stock IL 2975 which had been derived from IL 2856 in goat number 4 (see Fig. 4). The infection rate in tsetse probed on day 30 was 22.3%. Six Boran steers were infected, each with 5 infected tsetse. Of the six cattle, two animals (D-584 and D-587) had been injected with 0.5 mg kg⁻¹ isometamidium 30 days prior to the tsetse-transmitted infection; the remaining 2 animals (E-92 and E-93) served as the untreated controls. Full details of the experiment are given in Fig. 5 and are summarised below:

**Animals D-584 and D-587**: Infection was detected 17 days following the infected tsetse feeds and both animals were treated with 0.25 mg kg⁻¹ isometamidium. The infection relapsed. Moreover, the infection in the two animals relapsed even after further administration of isometamidium at 0.5 mg kg⁻¹ and later at 1.0 mg kg⁻¹. The animals were then treated with 7.0 mg kg⁻¹ diminazene aceturate. Whereas in
Fig. 5. Sensitivity of *T. congolense* stock IL 2975 (Banankeledaga/83/CRTA/67) to the prophylactic and therapeutic dose of isometamidium chloride as well as therapeutic dose of diminazene aceturate in Boran cattle. (For explanations of the abbreviations, see Figs. 1 and 2.)

animal D-584 the infection relapsed 45 days after the drug administration, Boran D-587 remained aparasitaemic for 91 days when both animals were administered 10.5 mg kg\(^{-1}\) diminazene aceturate. The two Borans remained aparasitaemic for 63 days when the experiment was terminated. In the experimental Borans D-585 and D-618 as well as in the control animals E-92 and E-93, the relapse of infection, the drug administration and the results were similar to those for Boran D-584.

Discussion

This study has shown that *T. congolense* stock IL 2466 which was originally isolated at Farakoba in 1978 was sensitive to a therapeutic dose of isometamidium chloride at 0.25 mg kg\(^{-1}\) and of diminazene aceturate at 3.5 mg kg\(^{-1}\). However, *T. congolense* stock IL 2468 isolated at Samorogouan in 1982 was sensitive to the therapeutic action of diminazene aceturate at the same dosage but resistant to both the prophylactic as well as to the therapeutic dose of isometamidium. In contrast, *T. congolense* stock IL 2856 isolated at Banankeledaga in 1983 was highly resistant to the therapeutic dose of diminazene aceturate, as well as to the prophylactic and
therapeutic dose of isometamidium. Thus, it would appear that the resistance to both these trypanocides had developed in *T. congolense* in Zebu cattle in the Bobo-Dioulasso region of Burkina Faso by 1983.

The infection rates in tsetse of the drug-resistant *T. congolense* stocks was as high as of the drug-sensitive stock. Moreover, the resistance to these two trypanocides remained stable after cyclical development in the tsetse vectors. These observations confirm the results of previous studies on populations of *T. vivax* and *T. congolense* isolated in Nigeria (Gray and Roberts, 1971a). Gray and Roberts (1971b) also found that the drug-resistant *T. vivax* stock retained this trait even after sequential tsetse-transmission of the parasite to four duikers and a gazelle. The present study has also shown that the rate of transmission of infection to mice by the infected tsetse was quite high both for *T. congolense* IL 2468 which is resistant to isometamidium but sensitive to diminazene aceturate, as well as IL 2856 which is resistant to both drugs. Besides, these tsetse became infected by feeding on cattle in which the infection had relapsed after treatment.

This study has thus demonstrated that resistance to the two most widely used drugs against cattle trypanosomiasis, namely isometamidium chloride and diminazene aceturate, had developed in *T. congolense* in the Bobo Dioulasso region of Burkina Faso. This trait is known to be stable for a long time and therefore such stocks can spread to wider areas through cattle movement and/or the spread of tsetse populations. There is therefore considerable need for continued experimental work in the field as well as under laboratory conditions to monitor the development of drug resistance in pathogenic trypanosome species.

**Acknowledgements**

We are grateful to Dr. A. Peregrine and Dr. R. Kaminsky for helpful comments on the manuscript. We thank Mr. I. Okumu and Mr. C. Sabwa for technical assistance, and Miss Catherine Barua for typing the manuscript and preparing the figures. This is ILRAD publication number 749.

**References**


