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Berenil (diminazene aceturate)-resistant *Trypanosoma congolense* in cattle under natural tsetse challenge at Kibaha, Tanzania

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Summary

Twenty-nine cattle, naturally infected with *Trypanosoma congolense* Kibaha, were subjected to chemotherapy with diminazene aceturate (Berenil, Hoechst) at 3.5 to 14.0 mg/kg. Fourteen animals recovered while six were refractory to treatment at 7.0 to 14.0 mg/kg. Further treatment of the Berenil-resistant isolates with isometamidium chloride (Samorin, May and Baker) at 1.0 mg/kg, effected cure. Corresponding chemotherapeutic trials in mice showed that the isolates were resistant to diminazene aceturate at 56.0 mg/kg and sensitive to Samorin at 20.0 mg/kg. It is noted, that *T. congolense* infections that do not respond to treatment with Berenil at 7.0 mg/kg may indicate development of resistance; the use of Samorin at 1.0 mg/kg or Homidium may be the alternative. The paper calls for judicious use of Berenil and Samorin, as they are the only sanative pairs available for the chemotherapy of bovine trypanosomiasis.

Key words: cattle; mice; trypanosomes; chemotherapy; Berenil; Samorin; drug resistance.

Introduction

Bovine trypanosomiasis ranks second to East Coast Fever as a major constraint toward livestock development in Tanzania (Mbwambo et al., 1986). About 74,818 head of cattle died of trypanosomiasis in the last five years (1982–1986). Trypanosomiasis is enzootic in most parts of the country, where tsetse abound. About 70% of Tanzania is infested by tsetse (Murray and Gray,

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1984). Three major species of salivarian trypanosomes (*Trypanosoma congolense, T. vivax* and *T. brucei*), transmitted cyclically, by different main species of tsetse (*Glossina morsitans, G. swynnertoni, G. pallidipes, G. palpalis, G. brevipalpis, G. austeni*) are involved in the epizootiology of the disease. Bush clearing to control the vectors (tsetse) and chemotherapy and/or chemoprophylaxis, are the main methods used for controlling animal trypanosomiasis in the country.

The efficacy of Berenil in the treatment of *T. congolense* infections in cattle, in Tanzania, dates back to the mid-fifties (Milne et al., 1955). Observations made on dairy cattle maintained by regular herd treatment with diminazene aceturate 3.5 mg per kg, at 14–20 day interval, for three successive years, showed no evidence of drug resistance (Wiessenhutter, 1967). However, work by Njau et al. (1983) reported on a resistant strain of *T. congolense,* isolated from goats, in Tanga, Tanzania. The strain manifested high mortality for mice, low mortality for sheep and goats and was not fatal to cattle. Observations made on a strain of *T. congolense*, Ngerengere, Tanzania, showed that the parasite was resistant to homidium chloride 1.0 mg per kg and Berenil 3.5 mg per kg but sensitive to the latter drug at 7.0 mg per kg (Anon, 1986). Nevertheless, as far as the treatment of cattle trypanosomiasis with Berenil and Samorin is concerned, no widespread resistance problems have been reported in Tanzania. However, there is an indication, that drug resistance could pose a serious impediment to effective control of animal trypanosomiasis.

The present work was provoked by high *T. congolense* infection rates at the Kibaha Education Centre Dairy Farm, recorded after treatment with diminazene aceturate 3.5 mg per kg. Its purpose was to investigate on possible *T. congolense* resistance to Berenil treatment.

Materials and Methods

Site and animals: In vivo drug trials were done on 29 grade cattle, naturally infected with *T. congolense*, at Kibaha Education Centre (KEC), 40 km away from Dar es Salaam, along the Tanzania Zambia Highway. KEC dairy farm is surrounded by bushes with medium tsetse challenge. Examination and identification of trypanosomes and drug sensitivity studies in mice, were carried out at ADRI Dar es Salaam.

Isolation of trypanosomes: Trypanosomes from each of the 6 cattle (among the 29 naturally infected) with Berenil-resistant suspected *T. congolense* strains, were isolated by intraperitoneal inoculation of 1.0 ml of blood in sodium citrate, into mice. The mice were subsequently bled from tail tip, and wet film examined under low power magnification. Examination started from day 4 post inoculation and treatment commenced 4 days after the mouse was parasitaemic (considered early stage treatment).

Drugs and treatments: Diminazene aceturate (4, 4-diamidino-diazoaminobenzene-diacetamidoacetate, Berenil, Hoechst and isometamidium chloride, Samorin, May and Baker) were used. Diminazene aceturate was used to treat *T. congolense* in cattle. It was injected deep into the gluteal muscles at various dosages (3.5, 7.0, 10.5 or 14.0 mg/kg in amounts not exceeding 20.0 mls per injection site. Following unsuccessful results, isometamidium chloride was injected deep into the same muscles at 0.5 or 1.0 mg/kg. In other trials, 12 *T. congolense* infected mice (2 per isolate) were treated with diminazene aceturate, subcutaneously, at various dosages (3.5, 5.0, 7.0, 10.5, 14.0, 28.0 or 56.0 mg/kg). Treatments at higher dosage rates were executed after failure of response to treatment

Table 1. The response of *Trypanosoma congolense* infections in cattle with Diminazene aceturate and Isometamidium chloride

Ear tag No	Sex	Weight (kg)	Initial T°C	Initial parasit-aemia	Maximum Berenil dose* (mg/kg)	Response	Maximum Samorin dose** (mg/kg)	Response
783	♂	450	40.5	+++	14.0	Resistant	1.0	Recovered
4336	♂	150	40.3	+++	14.0	Resistant	1.0	Recovered
4156	♀	300	40.6	+++	14.0	Resistant	1.0	Recovered
5065	♀	300	40.0	++	14.0	Resistant	1.0	Recovered
3917	♀	300	40.5	++	14.0	Resistant	1.0	Recovered
4212	♀	250	39.9	++	14.0	Resistant	1.0	Recovered

++ 1–10 parasites per microscope field
+++ above 10 parasites per microscope field
* other dosage rates tested included 3.5, 7.0 and 10.5 mg/kg
** other dosage rate tested was 0.5 mg/kg

during previous drug administration at lower dosage rates. Following failure of response to treatment with Berenil (death of mice due to *T. congolense*), the animals were treated with isometamidium chloride, injected into the muscles of the thigh at various dosages (0.5, 1.0, 2.0, 4.0, 8.0, 16.0 or 20.0 mg/kg). Twelve *T. congolense* infected mice were kept as controls.

Parasitaemia and trypanosome identification: Presence of parasites was checked by the micro-haematocrit/buffy coat technique, described by Murray et al. (1977), while identification of trypanosomes was based on morphological characteristics as described by Hoare (1970). Thick blood smears were examined under ×400 magnification while thin smears were examined under oil immersion, ×1000 magnification. Parasitaemia in thick blood smears was graded from "rare" (< than 1 parasite per microscope field), "numerous" (1–10 parasites per field) and "very numerous" (above 10 parasites per field).

Blood smears were taken from peripheral ear vein of cattle, fixed in methanol and stained with Giemsa as described by Killick-Kendrick (1968). Sampling was done just before treatment, and 48 h post treatment; then fortnighty until the case was confirmed cured or resistant by examination of buffy coat and mouse inoculation. Examination of wet blood films from tail tip of mouse was done daily for 5 days after treatment and then on alternate days until death.

Results

Isolation of T. congolense in mice: Prepatent period of the isolates in mice ranged from 18 to 46 days.

Chemotherapy trials in cattle: Out of the 29 cattle treated with diminazene aceturate, nine (31.0%) responded positively to 3.5 mg/kg; 14 cattle (48.3%) to 7.0 mg/kg (and 6 [20.7%] were refractory to 3.5 to 14.0 mg/kg, exhibiting persistent parasitaemia). Rectal temperatures fluctuated between 39.0 and 40.6°C. Milk production was very much affected (75% drop during clinical infection). However, the parasites were later cleared, following treatment with isometamidium chloride 1.0 mg/kg (Table 1), after failure to clear trypanoso-

mes at 0.5 mg/kg. Mice inoculation of blood from the cured animals showed no parasites, 50 days after inoculation.

Chemotherapy trials in mice: T. congolense isolates from the six Berenil-resistant suspected cases, were refractory to diminazene aceturate at 5.0 to 56.0 mg/kg. Examination of wet mounts 24 h after treatment onwards did not show decrease on the level of parasitaemia; parasites increased in number until death. Depending on virulence of the isolate, the infected-treated mice died on day 8–50 after treatment whereas, infected-untreated mice died after 8–29 days of initial parasitaemia. Further treatments of the Berenil resistant isolates in mice (other animals) with isometamidium chloride effected cure at 20.0 mg/kg. No relapses were observed 60 days after treatment.

Discussion

The standard curative dose of diminazene aceturate for the treatment of susceptible strains of trypanosomes in cattle is 3.5 mg/kg. It has been urged that Berenil will not promote the development of resistant trypanosome strains even if sub-therapeutic doses are administered (Fussganger and Bauer, 1960; Bauer, 1962). However, a strain of *T. congolense* from the field that was syringe passaged in cattle, was made resistant to Berenil by the method of subcurative dosage and relapses, albeit with much difficulty (Whiteside, 1963). During the present field trials, a cattle strain of *T. congolense* resistant to diminazene aceturate at 3.5 to 14.0 mg/kg, was recorded. It is probable that the resistance may have occurred as a result of the use of sub-therapeutic doses of Berenil and/or intensive use of the drug.

Isometamidium chloride and diminazene aceturate are prescribed as sanative pairs in the control of bovine trypanosomiasis in Tanzania, since neither induces cross resistance upon the other. The present findings are in agreement with this premise. Although *T. congolense* Kibaha, showed a high degree of resistance to Berenil, the parasites were cleared with isometamidium chloride at 1.0 mg/kg. Six cattle (20.7%) did not respond to Berenil at the recommended dose and twice the therapeutic dose. It appears that doses beyond 7.0 mg per kg may not be useful in the treatment of poorly responding trypanosome infections at normal therapeutic doses (3.5–7.0 mg/kg); may indicate development of resistance. Isometamidium chloride at 1.0 mg/kg or chemically related drugs (Homidiums) may be used to clear infection. Considering the price of Berenil, it is also uneconomical to resort to the drug at higher dosages than 7.0 mg/kg, where the sanative pairs (Samorin or Homidium) could suffice.

Corresponding results were obtained with Berenil in mice. The isolates were resistant to Berenil at dosages ranging from 5.0 to 56.0 mg/kg. Apart from postponement of death, difference on efficacy between single doses and two doses of 48 h apart were not noticed. The minimum curative dose of Berenil for

T. congolense in mice ranges from 5.0 to 20.0 mg per kg, depending on strain (Silayo, 1986). It has been proved that the minimum curative dose of diminazene aceturate for *T. congolense* in mice, is proportional to the infecting dose of trypanosomes (Walker and Opiyo, 1973). Treatment of *T. congolense* infection in mice was initiated at an early stage (4 days after the mouse was parasitaemic) of the infection to take care of this variable.

In view of the emergence of resistant strains of *T. congolense,* it is stressed that judicious use of Berenil and Samorin (sanative pairs) be exercised in order to avoid problems of drug resistance. It is further recommended that isometamidium chloride or the Homidiums be used where diminazene aceturate 7.0 mg per kg fails to clear *T. congolense* infections. This may help in delaying development of resistance.

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