TRYPANOSOMIASIS CHEMOTHERAPY: FURTHER OBSERVATIONS ON A STRAIN OF *TRYPANOSOMA CONGOLENSE*, RESISTANT TO DIMINAZENE ACETURATE.

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SUMMARY

Twenty nine cattle, naturally infected with *Trypanosoma congolense kilifia*, were subjected to chemotherapy with diminazene aceturate at four different dosages (3.5, 7.0, 10.5 and 14.0 mg per kg). Nine animals responded positively to 3.5 mg per kg., 14 at 7.0 mg per kg. and 6 were refractory to treatment at 3.5 to 14.0 mg per kg. Further treatment of diminazene aceturate-resistant strains with isometamidium chloride 1.0 mg per kg., effected cure in all animals. Corresponding chemotherapeutic trials in mice showed that the isolate was resistant to diminazene aceturate 5.0, 7.0, 10.5, 14.0, 28.0 and 56.0 mg per kg. b.wt. The present studies show that doses of diminazene aceturate above 7.0 mg per kg. may not be useful in the chemotherapy of Berenil-resistant strains of *T. congolense*; alternatively, isometamidium chloride 1.0 mg per kg. could be used to clear infection. Corresponding chemotherapeutic trials of the isolate in mice with isometamidium chloride 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mg per kg b.wt. were characterized by relapses with ultimate death, whereas 20.0 mg per kg effected cure. The paper calls for judicious use of Berenil and Samorin, as they are the only curative pairs recommended in the chemotherapy of bovine trypanosomiasis.

INTRODUCTION

Bovine trypanosomiasis ranks second to East Coast Fever as a major constraint towards 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, about 70% of Tanzania is infested by tsetse (Murray, 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.

Studies on the efficacy of Berenil in the treatment of *T. congolense* infections in cattle, in Tanzania, date 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.* (1981) reported on a resistant strain of *T. congolense*, isolated from goats, in Tanga. The strain manifested high mortality for mice, low mortality for sheep and goats and was not fatal to positive control cattle (un-treated).

Observations made on a strain of *T. congolense*, at Ngerengere, 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, 1985). 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.

Like in Tanzania Berenil-resistant trypanosomes were also being reported in Kenya and Uganda. Willy and Glover (1956) found two *T. congolense* strains which relapsed to 3.0 mg per kg. These were strains which had been found previously to

be resistant to Antrycide. A Kenya strain of *T. congolense*, resistant to 2.0 mg per kg. Ethidium, 2.0 mg per kg. Prothidium and 4.4 mg per kg. Antrycide, was found cross resistant to Berenil 6.0 and 7.0 mg per kg, when subinoculated into a Fresian steer (Anon, 1967). Early work by Whiteside (1960) showed that cross resistance between Berenil and Antrycide compounds did not exist. Similar trials conducted in Uganda by Mwambu and Mayende (1971), reported a strain of *T. vivax* resistant to Berenil 3.5 and 7.0 mg per kg. The author further reported that cross resistance between Berenil and Novidium chloride did not exist. Berenil-resistant strains of *T. vivax* have not yet been reported in Tanzania.

MATERIALS AND METHOD

Animals

In vivo drug trials were done on 29 grade cattle, naturally infected with *T. congolense*, at Kibaha Education Centre (KEC). 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 Animal Disease Research Institute in Dar es Salaam.

Isolation of trypanosomes

Trypanosomes from six 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 a mouse. The latter was subsequently bled from tail tip, and wet film examined under low power magnification. Examination started from day 4 post inoculation and early treatment commenced 4 days after the mouse was parasitaemic.

Drugs and treatments

Diminazene aceturate (4,4-diamidino-diazoaminobenzene-diacetamidoacetate, (Berenil, Hoechst) and isometamidium chloride (8-m-amidinophenyl-diazoamino-3-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride, Samorin, May and Baker were used. Diminazene aceturate was used to treat *T. congolense* in cattle; was injected deep into the gluteal muscles at various dosages (3.5, 7.0, 10.5 and 14.0 mg per kg b.wt.) with amounts not exceeding 20.0 ml per injection site. Following unsuccessful results, isometamidium chloride was injected deep into the same muscles at two different dosages (0.5 and 1.0 mg per kg). In other trials, twelve *T. congolense* infected mice (two per isolate) were treated with diminazene aceturate, subcutaneously, at various dosages (3.5, 5.0, 7.0, 10.5, 14.0, 28.0 and 56.0 mg per kg). Following Berenil resistance, isometamidium chloride was injected into the muscles of the thigh at ten different dosages (0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 20.0, 24.0, 32.0 and 40.0 mg per kg). Two *T. congolense* infected mice per isolate and six clean mice were kept as controls.

Parasites and trypanosome identification

Presence of parasites was checked by the microhaemotocrit/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 x 400 magnification. Parasitaemia in thick blood smears was graded from 'rare' (< than one parasite per microscope field); numerous (one to 10 parasites per field) and 'very numerous' (above 10 parasites per field).

Blood smears were taken from the 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 fortnightly until the case was confirmed cured or resistant by examination of buffy coat and mouse inoculation. Examination of wet blood 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 treated cattle with diminazene aceturate, nine (31.0%) responded positively to

3.5 mg per kg. 14 cattle (48.3%) to 7.0 mg per kg and 6 (20.7%) were refractory to 3.5 to 14.0 mg per kg, exhibiting persistent parasitaemia. 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 per kg. (Table 1), after failure to clear trypanosomes at 0.5 mg per kg. b.wt. Mice inoculated with blood from the cured animals showed no parasites, 50 days after inoculation.

Further treatments of Berenil resistant cases with isometamidium chloride 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mg per kg. b.wt. were characterized by relapses with ultimate death which occurred after 24 h to 99 days of treatment depending on stage of infection during treatment, dosage rate used and resistance of the mouse against trypanosomes. Recovery was achieved at 20.0 mg per kg. b.wt. and no relapses were observed (Table 3).

DISCUSSION

The standard curative dose of diminazene aceturate for the treatment of susceptible strains of

Table 1: Treatment of *Trypanosoma congolense* Kibaha, in cattle with Berenil* and Samorin**

Ear tag No	Sex	Weight (kg)	Initial T°C	Initial parasitaemia	Maximum Berenil dose (mg/kg)	Response	Maximum Samorin dose (mg/kg)	Response
783	M	450	40.5	+++	14.0	Resistant	1.0	Recovered
4336	M	150	40.3	+++	14.0	"	1.0	"
4156	F	300	40.6	+++	14.0	"	1.0	"
5065	F	300	40.0	++	14.0	"	1.0	"
3917	F	300	40.5	++	14.0	"	1.0	"
4212	F	250	39.9	++	14.0	"	1.0	"

*Diminazene aceturate **Isometamidium chloride
++Numerous trypanosomes (one to 10 parasites per microscope field)
+++Very numerous trypanosomes (> 10 parasites per microscope field)

Chemotherapy trials in mice

T. congolense isolates from all six Berenil-resistant suspected cases, were refractory to diminazene aceturate 5.0 to 56.0 mg per kg. Decrease in the level of parasitaemia following treatment was not noted; instead parasites kept on increasing in number until death. Depending on severity of the isolate and resistance of the mouse, the infected treated mice died after 10 - 50 days of treatment (Table 2) whereas, infected untreated mice died after 8 - 29 days of initial parasitaemia. All the six clean mice survived the whole period of observation (75 days). trypanosomes in cattle is 3.5 mg per kg.

It has been argued 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 directly 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 3.5 to 14.0 mg per kg., has been recorded.

Table 2: Treatment of *T. congolense kibaha*, in mice with diminazene aceturate

Mouse No.	Weight (gm)	Initial parasit-aemia	Parasit-aemia at death	Maximum Berenil dose (mg/kg)	Remarks
783	20	++	+++	56.0	Died on Day 22
4336	20	++	+++	56.0	Died on Day 8
4156	20	++	+++	56.0	Died on Day 24
5065	20	++	+++	56.0	Died on Day 50
3917	20	++	+++	56.0	Died on Day 46
4212	20	++	+++	56.0	Died on Day 16

++Numerous trypanosomes)
+++Very numerous trypanosomes) in wet mounts

Table 3: Treatment of *T. congolense kibaha*, in 20 g mouse with isometamidium chloride

Dosage (mg/kg)	Initial parasitaemia	Response to treatment after 24 h	48 h	72 h	96 h	120 h	Resurgence (Day)	Remarks
0.5	++	+++	+++	Died	NA	NA	NA	Dosage used
	+++	Died	NA	NA	NA	NA		insufficient
1.0	++	++	(++)	+	+	++	NA	Died on D 24
	+++	+++	(+++)	++	++	+++	NA	Died on D 24
2.0	++	++	(++)	(++)	(++)	++	NA	Died on D 12
	+++	+++	(+++)	(+++)	(+++)	+++	NA	Died on D 11
4.0	++	++	++	+	0	0	3 days later	Died on D 12
	+++	+++	(+++)	+++	0	0	3 days later	Died on D 12
8.0	++	++	+	+	0	0	5 days later	Died on D 61
	+++	+++	++	+	0	0	8 days later	Died on D 52
16.0	++	++	+	0	0	0	D 21	Died on D 99
	+++	+++	++	0	0	0	D 36	Died on D 62
20.0	++	+	0	0	0	0	Nil	Cured on D 60*
	+++	++	+	0	0	0	Nil	Cured on D 65
24.0	++	+	0	0	0	0	Nil	Cured on D 62
	+++	++	+	0	0	0	Nil	Cured on D 71
32.0	++	+	0	0	0	0	Nil	Cured on D 90
	+++	+	0	0	0	0	Nil	Cured on D 90
40.0	+++	+	0	0	0	0	Nil	Cured on 6 m
	+++	+	+	0	0	0	Nil	Cured on 6 m

\+ - Rare; ++ - Numerous; +++ - Very numerous
D - Day NA - Not applicable ( ) - Slight decrease in parasitaemia
* - Minimum period of observation (days)

Table 4: Trypanosomiasis chemotherapy and or chemoprophylaxis, 1970-1986*

Year	No. of cases	Chemotherapy	Chemoprophylaxis	Cured	Dead	% Mortality
1970	214,568	214,568	109,990	212,379	2,189	1.02
1971	219,377	219,377	183,193	216,707	2,670	1.22
1972	216,699	216,699	91,854	215,165	1,534	0.71
1973	128,408	122,579	101,902	119,062	1,317	1.03
1974	179,186	138,159	147,414	131,241	4,466	2.49
1975	152,828	152,828	15,159	149,980	2,848	1.86
1976	145,349	145,336	142,520	142,954	2,382	1.64
1977	139,496	139,496	122,385	135,179	2,292	1.67
1978	158,642	158,642	109,192	155,733	2,909	1.83
1979	155,292	146,692	92,545	15,744	3,552	2.29
1980	151,644	151,644	150,222	136,182	5,421	3.57
1981	121,391	120,274	112,213	112,671	7,603	5.27
1982	163,481	162,734	68,165	161,562	12,812	7.84
1983	117,418	117,418	60,731	110,253	7,165	6.10
1984	180,098	180,098	177,710	163,068	17,030	9.46
1985	159,962	136,930	200,234	131,299	28,663	17.92
1986	239,875	235,127	511,459	230,727	9,148	3.81
Total	2,720,021	2,676,922	2,527,166	2,441,512	113,645	4.18
Average	160,001.2	157,446.0	148,656.8	143,618.4	6,685.0	4.10

* - Minimum figures only; give an indication of what may be happening in the field.

It is suggested that the resistance may be a result of the use of subtherapeutic doses of Berenil and/or intensive use of the drug. Persistence of parasitaemia was a common feature and rectal temperatures fluctuated between 39.0 and 40.6°C. Milk production was much affected (75% drop during the clinical infection).

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 the argument. Although *T. congolense kibaha*, showed a high degree of resistance to Berenil, the parasites were cleared with isometamidium chloride 1.0 mg per 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 Berenil resistant strains of *T. congolense*; instead, isometamidium chloride 1.0 mg per kg. may be used. Considering the price of Berenil, it is also uneconomical to resort to the drug at higher dosages than 7.0 mg per kg where Samorin or Homidium could suffice.

It is noted that the intervals between samplings were long (fortnightly) because of lack of transport. Considering that the six cattle showed persistent parasitaemia, the likelihood of re-infections are thought not to have occurred. Since treatment with Berenil was almost fortnightly, and at higher dosages, any susceptible strains of *T. congolense* ought to have been cleared immediately. The question of cross resistance between Berenil and Homidium and Antrycide compounds is not feasible (Whiteside 1960; Mwambu and Mayende 1971).

Corresponding chemotherapy trials with Berenil in mice were equally bad. The isolates were resistant to Berenil at dosages ranging from 5.0 to 56.0 mg per kg. Apart from postponement of death, difference on efficacy between single doses and two doses of 48 h apart were not noticed. The fact that infected-treated and infected untreated mice died after 10 - 50 and

8 - 29 days of treatment respectively, further testifies that Berenil was useless in the present trials and that a variation on the degree of resistance among the mice does exist. In a series of experiments on the susceptibility of different inbred mice to *T. congolense* by Whitelaw *et al.* (1980), it was found that strains of mice differed markedly in their susceptibility to trypanosomiasis. 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 particularly initiated at an early stage (4 days after the mouse was parasitaemic) of the infection to take care of this factor.

Chemotherapy trials of the isolates, in mice, with Samorin were characterized by relapses that culminated in death at dosages below 16.0 mg per kg, and high efficacy at 20.0 mg per kg. Dosages at 1.0 and 2.0 mg per kg showed slight reduction in parasitaemia (lasting 24 and 72 h, respectively) after 48 h of treatment and then shot up until death. Differences/similarities between reduction/disappearance of parasitaemia and duration of illness following treatment at different levels of parasitaemia (Table 3) may have happened as a result of differences in mice susceptibility to trypanosome infection, among other factors. Resurgence of the infection was noted in dosages between 4.0 and <20.0 mg per kg. b.wt., indicating that the amount of drug used was not enough to effect cure. On the contrary resurgence was not seen at dosages of 20.0 mg per kg. and above (24.0, 32.0 and 40.0). These dosages were applied to see whether a quicker effect could be achieved and that the mouse could tolerate such high dosages or not. Disappearance of parasites was noted within 48 to 72 h of treatment, comparable to the results at 20.0 mg per kg. Problems in absorption of the drug due to inflammation were not noted. The period of observation was up to 6 months.

CONCLUSION

Realizing great losses of cattle due to trypanosomiasis (about 7000 head of cattle annually, 1970 - 1986, Table 4) and 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 Homidium be used where diminazene aceturate 7.0 mg per kg. fails to clear *T. congolense* infections.

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