Sensitivity to diminazene aceturate and isometamidium chloride of trypanosomes isolated from dogs in Nsukka area, Nigeria

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Key words

Dog - *Trypanosoma brucei* - *Trypanosoma congolense* - Drugs - Resistance to chemicals - Nigeria.

Summary

Trypanosome sensitivity to diminazene aceturate (Berenil) and isometamidium chloride (Samorin) was evaluated at two dose levels each (7 and 14 mg, and 0.25 and 0.5 mg/kg body weight, respectively) in mice infected by syringe. The trypanosomes had been isolated from clinically infected dogs taken to the University of Nigeria Veterinary Teaching Hospital (UNVTH), Nsukka, Enugu State, Nigeria. Six and eight of the 11 infected blood samples tested (10 with *Trypanosoma brucei*, one with mixed *T. brucei* and *T. congolense*) contained parasites that expressed various levels of resistance to Berenil and Samorin, respectively. Thus, three and five infected blood samples contained parasites that were considered to have low levels of resistance to Berenil and Samorin, respectively, while three samples each contained parasites that expressed moderate to high levels of resistance to the trypanocides. This trypanosome resistance to standard treatment doses of trypanocides portends serious danger to an effective chemotherapy of trypanosomosis in dogs of the area.

■ INTRODUCTION

Trypanosomosis due to *Trypanosoma brucei* and *T. congolense* is a major health problem of dogs in the Nsukka area of Nigeria (10). The clinical and pathological manifestations of the disease have been described in detail (4, 7). Currently, drug control of trypanosomosis is hampered by the emergence of parasites resistant to standard trypanocides (2).

The aim of this study was to evaluate the sensitivity of trypanosomes isolated from dogs in the Nsukka area to the standard recommended doses of diminazene aceturate and isometamidium chloride, the two most widely used trypanocides in the area (1).

■ MATERIALS AND METHODS

Infected blood samples were collected from clinical cases presented to the University of Nigeria Veterinary Teaching Hospital (UNVTH) between August 1992 and March 1994. Blood examination for trypanosomes was done by wet blood film, hematocrit buffy coat examination (8) and Giemsa-stained thin film. Trypanosome species were identified by their morphological characteristics on Giemsa-stained thin blood film preparations (12). The infection and treatment method was essentially used as described by Williamson and Stephen (14) to characterize the drug sensitivities of the isolated trypanosomes. Each infected blood sample was tested using 14 mice infected intraperitoneally (i.p.) with 10 µl of saline diluted with freshly-collected infected dog blood containing 10^5 trypanosomes. The mice were kept in clean cages in a fly-proof house, and feed and water were supplied *ad libitum*. On day five postinfection, when parasitemia was established based on wet film and hematocrit buffy coat examination (8), they were divided into three treatment groups. In group 1 two mice served as control; in group 2 six mice were treated with diminazene aceturate (Berenil), three at a dose of 7 mg/kg i.p. and three at 14 mg/kg i.p.; and in group 3 six mice were similarly divided, but treated with isometamidium chloride (Samorin) at doses of 0.25 and 0.5 mg/kg. Thereafter, the tail blood of the mouse was examined weekly for parasitemia until day 60 posttreatment (14) by the wet film and buffy coat technique. If parasitemia disappeared completely (i.e., no relapse), then the parasite was regarded as susceptible, while it was considered resistant if after treatment there was a relapse. The potency of the batches of diminazene aceturate and isometamidium chloride used was tested on a known drug sensitive organism.

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■ RESULTS

The prepatent period of infection was four to five days. The positive controls were readily sensitive to drug treatments thus proving the potency of the batches of trypanocide used. Eleven infected blood samples were examined comprising ten single *Trypanosoma brucei* and one mixed *T. brucei* and *T. congolense*. The trypanocidal susceptibility of the trypanosomes isolated are shown in table I. Relapses of the parasitemia were found in 8 of the 11 blood samples tested with one or both trypanocides used. Thus, there was a relapse in two blood samples that concerned the use of isometamidium chloride alone, and in six samples that concerned both diminazene aceturate and isometamidium chloride at different dose levels. With diminazene aceturate, relapse of parasitemia was observed in six samples, three at both the lower (7 mg/kg) and higher (14 mg/kg) dose levels, the remaining three at the lower dose level only. Similarly, parasitemia from three of the infected blood samples relapsed at both dose levels of isometamidium chloride (0.25 and 0.5 mg/kg), while that of the remaining five relapsed only at the lower dose level.

■ DISCUSSION

The results show that a considerable proportion of the studied trypanosomes resisted the therapeutic activities of diminazene aceturate and isometamidium chloride. The sensitivity of the trypanosomes to both trypanocides varied, with relapses occurring at different times and at various drug levels. Relapse of parasitemia at the lower drug doses was associated with a low level resistance of the parasites. On the other hand, parasites that resisted the higher doses were considered to be moderately to highly resistant to the drugs. Varying levels of drug sensitivity among trypanosome populations are known to affect the efficacy of chemotherapeutic agents (11).

The results further show that the application of the concept of a sanative pair—thus named because trypanosomes are not usually resistant to both trypanocides (13)—as a strategy to combat resistance in the area may be ineffective in view of the multiple resistance expressed by some of the trypanosomes to both diminazene aceturate and isometamidium chloride. Similar observations have been recorded by other workers from different parts of Africa (2, 5, 9). Furthermore, with a significant proportion of isolated trypanosomes expressing a high level of resistance to diminazene aceturate (the most common chemotherapeutic drug), the application of a high-dose treatment to forestall drug resistance (3) will be highly hazardous since diminazene aceturate has a narrow safety margin and is notably toxic in dogs (6).

■ CONCLUSION

It has been demonstrated that a number of trypanosomes (mainly *T. brucei*) isolated from clinically infected dogs presented to the UNVTH, Nsukka, Nigeria, resisted the normal therapeutic doses of diminazene aceturate and isometamidium chloride. This phenomenon poses a great danger to an effective chemotherapy of canine trypanosomosis in the area and highlights the need for a concerted effort on strategies to combat trypanocidal resistance before widespread treatments breakdown.

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Table I

Susceptibility of trypanosomes, isolated from dogs in Nsukka area, to diminazene aceturate and isometamidium chloride

TS	Drugs		Days between treatment and relapse - mean	Remarks
	Name	Dose (mg/kg body weight)		
Tb + Tc	Berenil	7.0	30	Low multiple resistance
		14.0	A	
	Samorin	0.25	16	
		0.5	A	
Tb	Berenil	7.0	A	Susceptible to Berenil and Samorin
		14.0	A	
	Samorin	0.25	A	
		0.5	A	
Tb	Berenil	7.0	53	Multiple resistance, moderate
		14.0	A	
	Samorin	0.25	19	
		0.5	26	
Tb	Berenil	7.0	53	Multiple resistance, high
		14.0	58	
	Samorin	0.25	35	
		0.5	56	
Tb	Berenil	7.0	A	Susceptible to Berenil, resistant to Samorin, low
		14.0	A	
	Samorin	0.25	42	
		0.5	A	
Tb	Berenil	7.0	44	Multiple resistance, low
		14.0	A	
	Samorin	0.25	28	
		0.5	A	
Tb	Berenil	7.0	28	Multiple resistance, high
		14.0	55	
	Samorin	0.25	26	
		0.5	37	
Tb	Berenil	7.0	A	Susceptible to Berenil and Samorin
		14.0	A	
	Samorin	0.25	A	
		0.5	A	
Tb	Berenil	7.0	28	Multiple resistance, moderate
		14.0	56	
	Samorin	0.25	26	
		0.5	A	
Tb	Berenil	7.0	A	Susceptible to Berenil and Samorin
		14.0	A	
	Samorin	0.25	A	
		0.5	A	
Tb	Berenil	7.0	A	Susceptible to Berenil, resistant to Samorin, low
		14.0	A	
	Samorin	0.25	42	
		0.5	A	

TS = trypanosome species
A = aparasitemia
Tb = *Trypanosoma brucei*
Tc = *Trypanosoma congolense*

REFERENCES

1. ANENE B.M., OMAMEGBE J.O., 1987. Common diseases of dogs in Nigeria. *Zariya vet.*, **2**: 46-55.

2. AUTHIE E., 1984. Mise en évidence d'une résistance aux trypanocides parmi des souches de *Trypanosoma congolense* récemment isolées au Burkina. *Revue Elev. Méd. vét. Pays trop.*, **37** : 219-235.

3. BISWAS R.K., HUNTER A.G., 1993. Effect of stage of infection with *Trypanosoma evansi* on Cymelarsan therapy. *Trop. Anim. Health Prod.*, **25**: 223-224.

4. KAGGWA E., MUNYA W.K., MUGERA G.M., 1984. Pathogenicity of *Trypanosoma brucei brucei* in dogs. *Bull. Anim. Health Prod. Afr.*, 3**2**: 360-368.

5. KALU A.U., 1995. Studies on the chemotherapeutic control of animal trypanosomiasis in Nigeria. *Stud. Res. vet. Med*., 82-92.

6. LOSOS G.J., CROCKETT E., 1969. Toxicity of Berenil in dogs. *Vet. Rec.*, **85**: 196.

7. MORRISN W.I., MURRAY M., WHITELAW D.D., SAYER P.D., 1983. Pathology of infection with *Trypanosoma brucei*: disease syndromes in dogs and cattle resulting from severe tissue damage. In: Gigase P.L., van Marok E.A.E., Eds. From parasitic infection to parasitic disease. *Contrib. Microbiol. Immunol.*, **7**: 103-119.

8. MURRAY M., MURRAY P.K., MCINTYRE W.I.M., 1977. Improved parasitological technique for the diagnosis of African trypanosomiasis. *Trans. R. Soc. trop. Med. Hyg.*, **75**: 325-326.

9. NJOKU A.R., HEATH B.R., 1986. Multiple drug resistance in *Trypanosoma vivax* in Kenya. *Vet. Rec.*, **118**: 133-134.

10. OMAMEGBE J.O., ORAJAKA L.J.E., EMEHELU C.O., 1984. The incidence and clinical forms of naturally occurring canine trypanosomiasis in two veterinary clinics in Anambra State of Nigeria. *Bull. Anim. Health Prod. Afr.*, **32**: 23-29.

11. PEREGRINE A.S., KNOWLES G., IBITAYO A.I., SCOTT J.R., MOLOO S.K., MURPHY N.R., 1991. Variation in resistance to isometamidium chloride and diminazene aceturate by clones derived from a stock of *Trypanosoma congolense*. *Parasitology*, **102**: 93-100.

12. SOULSBY E.J.L., 1982. Helminths, arthropods and protozoa of domesticated animals, 7th ed. London, UK, Bailliere Tindall.

13. STEPHEN L.E., 1986. Trypanosomiasis, a veterinary perspective. Oxford, UK, Pergamon Press, p. 355.

14. WILLIAMSON J., STEPHEN L.E., 1960. A test for drug resistant trypanosomes in experimental testse fly challenge of cattle. *Ann. trop. Med. Parasitol.*, **54**: 366.

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Résumé

Anene B.M., Chukwu C.C., Anika S.M. Sensibilité à l'acéturate de diminazène et au chlorure d'isométamidium de trypanosomes isolés de chiens dans la région de Nsukka, Nigeria

La sensibilité de trypanosomes à l'acéturate de diminazène (Bérénil) et au chlorure d'isométamidium (Samorin) a été évaluée à deux doses différentes pour chacun des produits (respectivement 7 et 14 mg/kg de poids corporel, et 0,25 et 0,5 mg/kg de poids corporel) chez des souris infectées par voie intrapéritonéale. Les trypanosomes avaient été isolés de chiens cliniquement infectés, après qu'ils aient été amenés à l'hôpital de l'Ecole vétérinaire de l'Université du Nigeria à Nsukka, Etat d'Enugu, Nigeria. Sur les 11 échantillons sanguins infectés testés (10 par *Trypanosoma brucei* et un seul à la fois par *T. brucei* et *T. congolense*), six et huit contenaient des parasites qui ont présenté des niveaux de résistance différents respectivement au Bérénil et au Samorin. Ainsi, trois et cinq échantillons sanguins infectés contenaient des parasites considérés comme ayant un faible niveau de résistance respectivement au Bérénil et au Samorin, alors que trois échantillons pour chacun des produits contenaient des parasites présentant des niveaux de résistance aux trypanocides modérés à importants. Cette résistance des trypanosomes aux doses standard de traitement par les trypanocides laisse présager de sérieux problèmes lors de la réalisation d'une chimiothérapie efficace contre la trypanosomose chez les chiens de cette région.

Mots-clés : Chien - *Trypanosoma brucei* - *Trypanosoma congolense* - Médicament - Résistance aux produits chimiques - Nigeria.

Resumen

Anene B.M., Chukwu C.C., Anika S.M. Sensitividad al aceturato de diminaceno y al cloridio de isometamidio de tripanosomas aislados en perros en Nsukka, Nigeria

Se evaluó la sensitividad al aceturato de diminaceno (Berenil) y al cloridio de isometamidio (Samorin), ambos a dos niveles diferentes de dosis (7 y 14 mg y 0,25 y 0,5 mg/kg de peso corporal, respectivamente), en ratones infectados mediante una jeringa. Los tripanosomas fueron aislados en perros infectados clinicamente, presentes en el Hospital de Enseñanza Veterinaria de la Universidad de Nigeria (UNVTH), Nsukka, Estado de Enugu, Nigeria. Seis y ocho de las 11 muestras de sangre infectada que se estudiaron (10 con *Trypanosoma brucei*, una con una mezcla de *T. brucei* y *T. congolense*) contenían parásitos que presentaron varios niveles de resistencia al Berenil y al Samorin, respectivamente. Por lo tanto, tres y cinco muestras de sangre infectada contenían parásitos que fueron considerados como poseedores de un nivel bajo de resistencia al Berenil y al Samorin, respectivamente, y tres muestras de ambos contenían parásitos que presentaron niveles de resistencia de moderados a altos a los tripanocidas. Esta resistencia del tripanosoma a dosis terapéuticas estándar de tripanocidas presagia un serio peligro para la quimioterapia efectiva de la tripanosomosis en perros en el área.

Palabras clave: Perro - *Trypanosoma brucei* - *Trypanosoma congolense* - Medicamento - Resistencia a productos químicos - Nigeria.