

Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 44 (1987)
Heft: 3

Artikel: Adaptation of "Trypanosoma congolense" stocks to in vitro culture does not change their sensitivity to isometamidium : short communication
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DOI: <https://doi.org/10.5169/seals-313865>

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Adaptation of *Trypanosoma congolense* stocks to in vitro culture does not change their sensitivity to isometamidium

Short communication

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Recent advances in trypanosome culture techniques have resulted in systems in which bloodstream forms can be grown in vitro to test known and potential trypanocidal drugs (Borowy et al., 1985; Hawke, 1985). Such cultures can contribute to the development of rapid screening procedures for identification of new trypanocides and can also be used in the identification and characterization of drug resistant trypanosome stocks.

However, it is important in such experiments to ensure that the natural characteristics of the parasites are altered as little as possible when maintained in vitro. Prior to performing in vitro experiments on stocks of *Trypanosoma congolense* which differ in their sensitivity to the trypanocide isometamidium chloride (Samorin, May and Baker), it was considered necessary to ensure that adaptation of trypanosomes to culture conditions did not affect their drug sensitivity.

Materials and Methods

Trypanosome stocks. Three stocks of *T. congolense* were used. TREU 1627 and TREU 1467 had been previously adapted to culture (Ross et al., 1985), and a third, GRVPS 8, more recently. Culture forms of these three stocks were re-established in mice and stabilised as GRVPS 38, GRVPS 41, and GRVPS 32, respectively. All stabilates made after adaptation of trypanosomes to culture, and which were tested for drug sensitivity, were derived initially from infective cultured metacyclic forms.

Drug treatment. Initial experiments to test all three original blood stocks for isometamidium sensitivity were performed in groups of at least 5 outbred CD1 mice (Charles River, Margate). Mice were infected with 1×10^4 TREU 1627 or TREU 1467 or 1×10^5 GRVPS 8 by intraperitoneal (i/p) injection. Mice were treated, also i/p, at peak parasitaemia. The doses of drug used were as follows: 0.001 mg/kg, 0.01 mg/kg, 0.1 mg/kg, 1 mg/kg, 5 mg/kg and 10 mg/kg. The minimum curative dose (MCD) of isometamidium for each stock, i.e. the lowest dose which successfully cured mice of infection, was 5 mg/kg for TREU 1467 and GRVPS 8 and 0.1 mg/kg for TREU 1627. In some experiments, one mouse of a group infected with TREU 1467 and treated with 5 mg/kg drug developed a relapse parasitaemia, but 10 mg/kg was always curative.

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Table 1. Isometamidium sensitivity of bloodstream forms of *T. congolense* before and after adaptation to culture

	Trypanosome stock		Dose of isometamidium (mg/kg)							
	before culture	after culture	0	0.001	0.01	0.1	0.5	1.0	5.0	10.0
1.	TREU 1627		5/5 ^a	ND ^b	5/5	0/5	ND	ND	ND	ND
		GRVPS 38	5/5	5/5	5/5	0/5	ND	0/5	ND	ND
2.	TREU 1467		5/5	ND	ND	ND	ND	5/5	1/5	ND
		GRVPS 41	5/5	ND	ND	5/5	ND	5/5	1/5 ^c	0/5
3.	GRVPS 8		5/5	ND	ND	5/5	ND	2/5	0/5	ND
		GRVPS 32	3/3	3/3	8/8	4/4	5/5	3/7	0/4	ND

^a All numbers represent number of mice developing a parasitaemia after treatment/total number of mice per group.

^b Not done.

^c One further mouse in this group displayed a low transient parasitaemia from 60 days after treatment.

The subsequent experiments to compare the MCDs of the trypanosome stocks both before and after culture were conducted using the same infection doses and treatment times.

Results and Discussion

The experimental protocol and results are presented in Table 1. It is apparent from Table 1 that the MCD for all three stocks was the same whether or not the trypanosomes had been adapted to in vitro culture. It is therefore concluded that adaptation to culture, including completion of the entire life cycle in vitro, does not alter the sensitivity of these trypanosome stocks to isometamidium. It has also been demonstrated that transmission of trypanosomes through tsetse flies does not change their drug sensitivity (Gray and Roberts, 1971a, b).

Acknowledgments. This work was supported by grants from the O.D.A. and the Commission of the European Communities. H. C. Brown is a Research Fellow supported by the Animal Health Trust. We wish to thank Norma Cathcart for technical assistance.

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