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A RAPID METHOD FOR TESTING *IN VIVO* THE SUSCEPTIBILITY OF DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI* TO ACTIVE CHEMOTHERAPEUTIC AGENTS

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A method is described which permits to determine in vivo *and in a short period of time (4-6 hours) the sensitivity of* T. cruzi *strains to known active chemotherapeutic agents. By using resistant- and sensitive* T. cruzi *strains a fairly good correlation was observed between the results obtained with this rapid method (which detects activity against the circulating blood forms) and those obtained with long-term schedules which involve drug administration for at least 20 consecutive days and a prolonged period of assessment. This method may be used to characterize susceptibility to active drugs used clinically, provide information on the specific action against circulating trypomastigotes and screen active compounds.*

Differences in the natural susceptibility of *Trypanosoma cruzi* strains to active drugs have been already reported using different criteria, mostly demanding long-term study of the animals (Hauschka, 1949; Bock, Gonnert & Haberkorn, 1969; Brener, Costa & Chiari, 1976; Andrade & Figueira, 1977; Schlemper, 1982). In this paper we report a method which detects in 4-6 hours the effect of drugs on bloodstream forms in mice with established *T. cruzi* infections. The results obtained with this method show a fairly good correlation with those obtained by prolonged treatment schedules used to assess the action of drugs in experimental Chagas' disease and may be used to study the sensitivity of *T. cruzi* strains to active drugs.

MATERIAL AND METHODS

Drugs used

3-methyl-4 (5'-nitrofurfurylidene-amino)-tetrahydro-4H-1, 4-thiazine-1, 1-dioxide (nifurtimox); N-benzyl-2-nitro-1-imidazolacetamide (benznidazole); 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1, 3, 4-thiadiozole (CL 64'855). In addition to those active compounds, we used as controls 2-acetamine-5-nitrothiazole (aminotrozol) and diethyl carbanazine ("Hetrazan") which have little if any activity in *T. cruzi* experimental infections. Nifurtimox and benznidazol are active compounds already used in humans (for review see Brener, 1979, Cançado & Brener, 1979). CL 64'855 is an extremelly active compound recently studied by Filardi & Brener (1982).

T. cruzi strains

CL and *MR* (Brener & Chiari, 1963), *Y* (Silva & Nussenzweig, 1953), *Colombiana* (Andrade & Figueira, 1977) and *VL-10* (Schlemper, 1982). Those strains are maintain-

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ed by syringe passages in the laboratory. The *Colombiana* and *VL-10* strains are highly resistant to prolonged treatment with nifurtimox and benznidazol.

Evaluation of drug activity by the rapid method

Male albino mice, 18-20g, were inoculated intraperitoneally with 1 x 10^5 blood trypomastigotes. At the peak of parasitemia (9-11 days with *CL* and *MR* strains, 7 days for *Y*; 15-20 days for *Colombiana* and 15-19 days for *VL-10*) a single dose of 500mg/kg of the drugs to be tested was given by oral route. The number of circulating bloodstream forms was determined according to Brener (1962), before and 2, 4, 6, 8 hours after drug administration. Untreated mice similarly inoculated were used as controls. The percentage of reduction of the parasitemia was calculated comparing the number of parasites obtained at each interval of time after drug administration with that found before treatment. For the study of dose-effect relationship 4 groups of mice inoculated with the *MR* strain received, respectively, a single dose of 500, 250, 125 and 62,5mg/kg of benznidazol. In another experiment mice immunosuppressed by X-ray irradiation (650r) have been inoculated with the *Y* strain and then treated with benznidazol. In all experiments the untreated group was included as control and the number of blood forms determined as for the treated groups.

Long-term treatment

Fifteen male albino mice, 18-20g, were inoculated intraperitoneally with 5 x 10^4 blood parasites. Twenty consecutive doses of 100mg/kg of drug was given by oral route, beginning 24 hours after inoculation. Untreated mice similarly inoculated were used as controls. Fresh blood examinations were performed at least twice after treatment. All mice were bled from the orbital sinus 30 days after treatment and about 0.2-0.3ml of blood inoculated into two tubes of LIT medium (Camargo, 1964). The hemocultures were maintained at 28°C and microscopically examined after 30 and 60 days.

RESULTS

Fig. 1 illustrates typical results obtained with nifurtimox and benznidazol in groups of mice inoculated with *T. cruzi* strains. A reduction of about 85% of the parasitemia was detected with the *MR* strain (drug-sensitive) whereas in the animals inoculated with the *VL-10* strain the number of circulating parasites was kept at the same level observed before treatment (drug-resistant strain). A clear dose-effect was detected in an experiment in which animals inoculated with the *MR* strain have been treated with different doses of benznidazol (Fig. 2).

Table I shows comparative results between the rapid method and the long-term schedule of treatment. In general a fairly good concordance was observed using different strains and active drugs. An exception was observed in the experiment number 9 in which a discrepancy was observed between the rather high reduction rate of bloodstream forms and the low percentage of cure achieved with the long-term schedule. Our present data confirm the remarkable activity of compound CL 64'855 which cured all treated animals (including those inoculated with the resistant strains *Colombiana* and *VL-10*) and induced the highest reduction rates of circulating parasites.

DISCUSSION

Brener (1971) was the first to investigate the susceptibility of *T. cruzi* bloodstream forms to active compounds. The author based his method on the fact that *T. cruzi* stout blood forms intravenously inoculated into mice persist for some hours in the bloodstream without penetrating the host tissues (Brener, 1969). Normal mice were then treated with compounds to be tested and next intravenously inoculated with stout forms

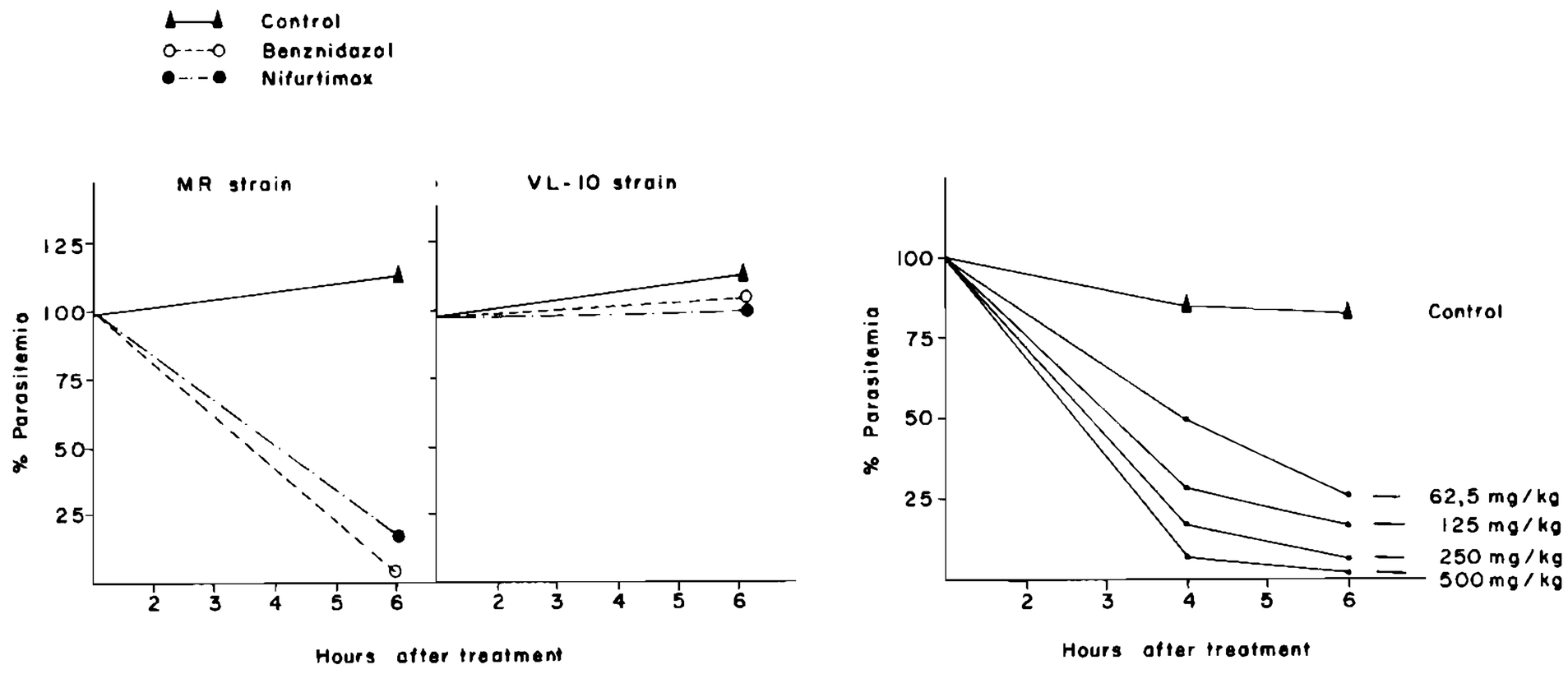

Fig. 1 – Percentage of parasitemia reduction in mice inoculated with *T. cruzi* strain *MR* and *VL-10*, treated by the rapid method with nifurtimox and benznidazol (500mg/kg, oral route). Fig. 2 – Dose-effect determination in mice inoculated with *MR T. cruzi* strain and treated with different doses of benznidazol.

TABLE I

Results obtained with the rapid method and long-term treatment schedule using three active drugs and different *T. cruzi* strains

No. of experiment	Long-term treatment				Rapid method	
	Strain	Drug	Dose mg/kg (20x)	% cure	Dose mg/kg (1x)	% reduction blood forms
1	Y	CL 64'855	100	100.0	500	100
2	Y	Nifurtimox	100	66.6	500	77
3	Y	Benznidazol	100	66.6	500	77.4 ± 9.6 *
4	MR	CL 64'855	100	100.0	500	100
5	MR	Nifurtimox	100	93.3	500	85
6	MR	Benznidazol	100	100.0	500	98
7	Colombiana	CL 64'855	100	100.0	500	100
8	Colombiana	Nifurtimox	100	0.0 **	500	31.6 ± 3.8*
9	Colombiana	Benznidazol	100	6.6 **	500	88.8 ± 8.7***
10	Colombiana	Aminotrozol	ND	ND	500	0
11	Colombiana	Hetrazan	ND	ND	500	0
12	VL-10	CL 64'855	100	100.0	500	91
13	VL-10	Nifurtimox	100	0.0	500	6
14	VL-10	Benznidazol	100	13.3	500	7
15	CL	CL 64'855	ND	ND	500	98
16	CL	Nifurtimox	100	93.3	500	78
17	CL	Benznidazol	100	100.0	500	88
18	CL	Hetrazan	ND	ND	500	0
19	Y	Benznidazol	ND	ND	500	76****

*mean of 3 experiments; **mean of 2 experiments; ***mean of 5 experiments; ****X-irradiated mice; ND – not determined

of the *MR* strain. Active drugs induced a rapid decline in the number of the blood parasites demonstrating the feasibility of a test which could detect drug activity just against circulating stages of the parasite. This method, however, could not be used with strains displaying predominance of slender forms which readily penetrate the host tissue after inoculation (Brener, 1969). On the contrary, the present method can be used with practically any *T. cruzi* test and does not involve the risky procedure of inoculating intravenously the trypomastigotes.

The correspondence between results obtained with the rapid method and the long-term treatment was rather high. The only significant discrepancy was the high reduction rate observed in mice inoculated with the *Colombiana* strain and treated with benznidazol, contrasting with the low percentage of cured mice with the prolonged treatment (experiment 9). Those data would suggest that benznidazol is more active against bloodstream forms of this strain than intracellular stages, a phenomenon likely to explain the persistence of infection after prolonged treatment. Docampo et al. (1981) demonstrated that the production of hydrogen peroxide by nifurtimox (a crucial event in the mechanism of toxicity of nitrofuran derivatives) occurs in all developmental stages of *T. cruzi* but is higher in amastigote than in epi- and trypomastigote stages. Benznidazol, however, in opposition of nifurtimox, does not generate superoxide anion and hydrogen peroxide by epimastigote cells or homogenates (Moreno et al., 1982). Since neither the mechanism of action of benznidazole nor its relative efficacy on the different *T. cruzi* stages are known, it is difficult to speculate on the results of our experiment. Peculiarities of the *Colombiana* strain related to differences in the drug absorption and/or detoxification between amastigote and trypomastigote stages could explain those data.

This method may be used to characterize very rapidly resistance of *T. cruzi* strains to drugs used clinically, a process, which, otherwise, would take a long time. By conventional methods this demonstration involves treatment for 20-30 days, assessment by hemocultures performed at least 30 days after the end of treatment examination of the hemocultures 30 days thereafter. Moreover, this method provides information on the specific action against the circulating bloodstream forms and may complement data relative to the effects of drugs on *T. cruzi* intracellular stages which can be gathered by studies on tissue culture or by electron microscopy (Maria, Tafuri & Brener, 1972). A handicap to this method would be the existence of low virulent strains which are unable to induce high parasitemias in mice. In this case the inoculation of the parasites in irradiated immunosupressed mice may overcome this problem since these animals respond to drug administration similarly to the normal hosts.

This technique might also represent a rapid screening method that offers results in short periods of time and requires small numbers of test animals.

RESUMO

No presente trabalho descreve-se um método que permite determinar *in vivo* e em curto espaço de tempo (4-6 horas) a sensibilidade de cepas de *T. cruzi* a agentes terapêuticos ativos na doença de Chagas. Usando-se cepas sensíveis e resistentes aos medicamentos foi possível observar uma boa correlação entre os resultados obtidos com o método rápido (que detecta atividade contra as formas circulantes do parasita) e aqueles obtidos com esquema de ação prolongada que envolve a administração da droga por 20 dias e posterior avaliação. Esse método pode ser usado para caracterizar a sensibilidade de cepas a drogas ativas usadas clinicamente, fornecer informações específicas sobre a ação medicamentosa em tripomastigotas sangüíneos e, eventualmente, para triagem de novos compostos.

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