Rev. Inst. Med. trop. São Paulo
13(4):302-306, julho-agôsto, 1971

UDC 593.161.13

STUDY OF THE ACTION OF SOME ACTIVE DRUGS AGAINST *TRYPANOSOMA CRUZI* BLOOD FORMS

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SUMMARY

Trypanosoma cruzi stout trypomastigotes intravenously inoculated in mice can persist, for some time, in the blood stream without penetrating the host's tissues. This fact is now used as a tool for studying *in vivo* drug action just against *T. cruzi* blood forms. Normal mice had been previously treated with different compounds and then intravenously inoculated with the parasites: a rapid decrease of the parasites is considered as a result of drug action against circulating parasites whereas persistence of the trypomastigotes indicates lack of action. This method may provide complementary information for the studies of drug action against *T. cruzi* intracellular forms.

INTRODUCTION

Many compounds have displayed marked supressive effect in *T. cruzi* experimental infections and the action of some such compounds on the intracellular forms of the parasite has been already investigated by various Authors using different tissue cultures infected with *T. cruzi* (SILVA & KIRCHNER [10]; BAYLES et al. [2]; BRENER [5]; MIETH & SEIDENHAT [9]). More recently BRENER et al. [8] performed an electron microscope study of *T. cruzi* intracellular parasites on mice experimentally infected and treated with an active nitrofuran compound, showing the sequence of alterations undergone by the parasite after drug administration. So far, no attempt has been made to investigate the direct action of active compounds just against the circulating blood forms. The present paper describes a method allowing the study of drug action on *T. cruzi* trypomastigotes which, after being intravenously inoculated on mice previously treated, persist in the blood stream for some time without penetrating the tissues and are, therefore, submitted to active concentrations of the drug in the host's blood.

MATERIAL AND METHODS

T. cruzi strain — "MR" strain, isolated from naturally infected *Triatoma infestans* colleted in Rio Grande do Sul, Southern Brazil (BRENER [4]). In animals inoculated with this strain, stout and broad blood trypomastigotes predominate and constitute most of the parasites after the 8th or 9th days of infection (BRENER [4]).

Drugs used

a) 5-nitro-2-furaldehyde semicarbazone (nitrofurazone, 100 mg/kg, p.o.)
b) 8 - (4-amino-1-methylbutylamino)-6-methoxy-quinoline (primaquine, 15 mg/kg, p.o.)
c) 3 - amino-9-p-carbethoxyaminophenyl-10-methyl-phenanthridinium ethano-sulphonate (carbidium sulphate, 15 mg/kg, s.c.)
d) diallylmalonyl-(4 - amino-2-methyl-quinolyl-6-amide) acetate ("3024 I.C.I.", 50 mg/kg, i.p.)
e) 1 - (3-dimethylaminopropyl)-4-(p-methoxyphenyl) piperazine dihydrochloride (piperamide, 100 mg/kg, p.o.)

This work has been supported by the Research National Council, Brazil
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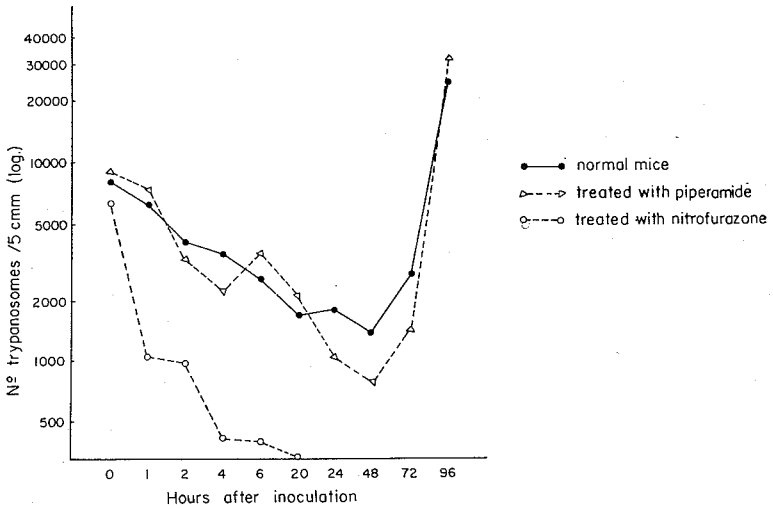

Fig. 1 — Course of parasitemia in mice treated with nitrofurazone and piperamide, as well as in untreated inoculation of *T. cruzi* blood forms ("MR" strain)

Groups of 5 albino mice weighing 20 g have been treated for 5 consecutive days and then inoculated, by intravenous route, with about 2,000,000 blood forms of "MR" strain. To this purpose, there have been killed animals previously infected with "MR" strain and showing a high percentage of stout trypomastigote forms, the blood being collected from their axillary vessels and treated with heparin. The number of parasites in the pooled blood and in the inoculated animals was determined according to a method described by BRENER [3]. The previously treated mice as well as the normal controls were intravenously inoculated in the tail and the parasites in the blood stream counted immediately after inoculation and, thereafter, at different intervals of time. After inoculation, treatment was performed for two days longer.

A group of animals inoculated with 100,000 blood forms of *T. cruzi* "Y" strain has been treated with 45 doses of piperamide (100 mg/kg), treatment starting on the day after inoculation.

RESULTS

Figure 1 shows the course of parasitemia in mice treated with nitrofurazone and piperamide as well as in untreated animals, after intravenous inoculation of the parasites. In the animals treated with nitrofurazone, there occurred a rapid and marked decrease in the number of injected trypomastigotes, indicating strong action of the drug against the circulating parasites. On the other hand, no decrease has occurred in the animals treated with piperamide, the parasitemia curve being quite similar to that from the untreated animals. Table I shows the results obtained through the administration of piperamide to a group of mice for 45 consecutive days. As can be observed, no activity has been detected on the first days of treatment, the number of trypomastigotes decreasing 10 days afterwards. Table II shows the results obtained with untreated controls.

Figure 2 shows the course of parasitemia in groups of animals treated with primaquine and carbidium sulphate as well as in untreated controls, after intravenous inoculation. Finally, Fig. 3 shows the course of parasitemia both in a group of mice treated with compound "3024 I.C.I." and in untreated controls after inoculation.

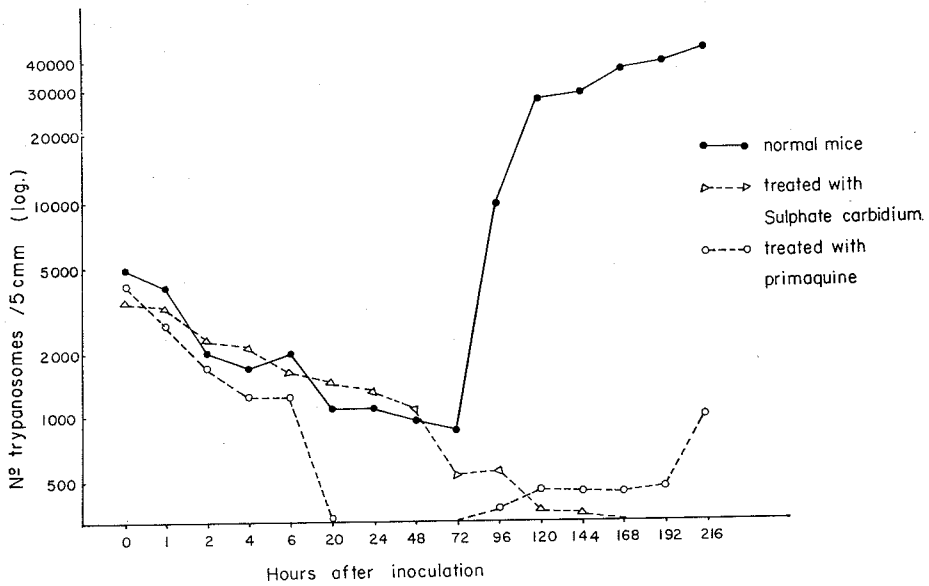

Fig. 2 — Course of parasitemia in mice treated with primaquine and carbidium sulphate, as well as in untreated controls, after intravenous inoculation of *T. cruzi* blood forms ("MR" strain)

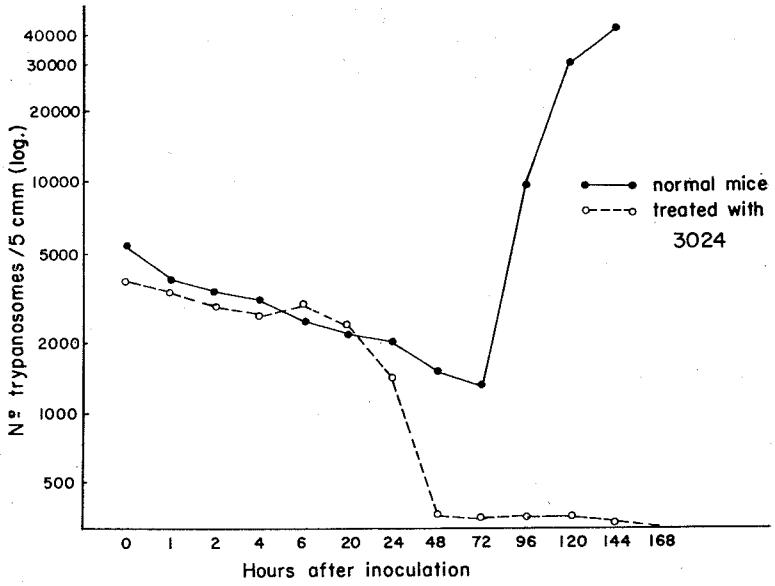

Fig. 3 — Course of parasitemia in mice treated with "3024 I.C.I." and in untreated controls, after intravenous inoculation of *T. cruzi* blood forms ("MR" strain)

DISCUSSION

The occurrence of *T. cruzi* strains showing a high predominance of stout trypomastigotes has been reported by BRENER & CHIARI [7] and BRENER [4]. In a recent paper, BRENER [6] mentioned that stout forms intravenously inoculated into normal mice could persist in the blood stream for some days without penetrating the tissues. This phenomenon has now been serving as a means of studying drug action against circulating *T. cruzi* blood forms. In previously treated animals, the rapid decrease of intravenously inoculated parasites was considered as a proof of drug action just against blood forms. Apparently, this method has some advantages over "in vitro" studies performed through procedures where drug metabolism and excretion are not present. Besides, it may provide complementary information for the interpretation of results obtained with other methods used for the assessment of drug activity against intracellular forms, such as tissue culture and electron microscope studies. It should be remarked, however, that such results have been obtained through experiments performed on a peculiar form of trypomastigote which may happen to have peculiar biological behaviour.

As regards the results obtained, the following conclusions were, then, reached: a) nitrofurazone is very active against the trypomastigote circulating forms, which are rapidly cleared from the blood stream, the afore mentioned marked action of nitrofuran compounds against intracellular forms (BRENER [5]; ANDRADE & BRENER [1]; BRENER et al. [8]) accounting for the further absence of trypomastigotes in the blood of the animals; b) piperamide is apparently inactive against the blood forms, at least in the first two weeks of drug administration (Table I). This unsual lag period, so far unexplained, is quite in accordance with the results obtained in the present experiments, which shows complete inactivity of the compound against the inoculated blood forms; c) primaquine also induces rapid decrease in the number of circulating parasites, a reccurring increase of parasitemia having however been detected on the 4th day after inoculation. Apparently, some parasites, after having accomplished the tissue cycle, emerged again in the blood. This fact seems to be in agreement with the findings of SILVA & KIRCHNER [10] who demonstrated, by means of infected tissue cultures, that primaquine has no effect on the intracellular parasites; d) "3024" and carbidium sulphate have shown to be less active compounds, the former drug, however, seeming to be actually active against the extracellular circulating forms. It is worth reminding that MIETH & SEIDENHAT [9] could not detect, using infected tissue cultures, any activity of "3024" (= "Bayer 7602 A.C.") against either intracellular or extracellular parasites.

TABLE I

Number of trypomastigotes in 5 c.mm. of blood in mice experimentally inoculated, by intraperitoneal route, with about 100,000 blood forms of *T. cruzi* and treated, from the day after inoculation on, with 45 consecutive doses of piperamide (100 mg/kg, p.o.)

no.	Days after inoculation												
	5	7	8	9	12	14	19	22	26	29	36	40	45
1	16100	16800	17500	5600	560	140	210	0	0	0	0	0	0
2	17500	9800	10500	2100	140	140	70	0	0	0	0	0	0
3	16800	9800	13300	4200	700	420	70	70	0	0	0	0	0
4	21000	10500	16100	5600	2380	1050	700	140	0	70	0	0	0
5	13300	12600	14000	7000	840	770	140	70	0	0	0	0	0
6	10500	8400	6300	6230	350	140	70	140	0	0	0	0	0
7	21000	2400	5600	4900	2870	700	70	0	0	0	0	0	0
8	17500	2100	7700	6300	280	980	420	70	0	0	0	0	0
9	19600	21000	16000	3500	1400	1610	210	70	0	0	0	0	0
10	18900	5600	10500	1050	dead								

TABLE II

Number of trypomastigotes in 5 c.mm of blood in mice experimentally inoculated, by intraperitoneal route, with 100,000 blood forms of *T. cruzi*

no.	Days after inoculation				
	5	7	8	9	12
1	16100	28000	16100	7700	dead
2	10500	25900	15400	6300	4900
3	11200	19600	10500	7000	dead
4	18900	21000	13300	4200	dead
5	14000	19600	10500	4900	dead
6	15400	42000	16100	3500	dead

RESUMO

Estudo da ação de algumas drogas ativas contra as formas sanguíneas do Trypanosoma cruzi

Formas largas do *T. cruzi*, inoculadas, por via endovenosa, em camundongos normais, são capazes de sobreviver no sangue circulante dos animais, por algum tempo, sem penetrar nos tecidos. Êsse fenômeno foi usado na elaboração de um método de estudo da ação, *in vivo*, de diferentes compostos sôbre formas circulantes do *T. cruzi*. Camundongos normais foram prèviamente tratados e, posteriormente, inoculados por via endovenosa: uma acentuada diminuição do número de parasitas no sangue aparentemente indica atividade contra formas circulantes ao passo que a sua persistência no sangue demonstra inatividade contra formas sanguíneas. Êsse método pode complementar os estudos da ação de compostos contra as formas intracelulares do *T. cruzi*.

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Recebido para publicação em 7/12/1970.