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TRYPANOCIDAL EFFECTS OF PROMETHAZINE

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Summary Promethazine is currently used for its antipsychotic and ansiolytic effects. It is a phenothiazine with anticalmodulin action, not toxic for human beings at therapeutic dosis. The present results show that promethazine has trypanocidal effect on both epimastigote and trypomastigote stages of *T. cruzi*; two hundred μM inhibited epimastigote growth in culture medium and 2 μM immobilized and killed bloodstream trypomastigotes. When promethazine (55 mg/Kg/day) was used as treatment of *T. cruzi* infected mice, it proved effective in reducing parasitemia and it increased the survival of treated animals. Ultrastructural studies suggest that the lethal effect of this phenothiazine is related to a detergent effect that disrupts *T. cruzi* cell membrane.

Key words: *Trypanosoma cruzi*, Chagas' disease, promethazine (PRO), trypanocidal drugs

One of the greatest health problems in Latin America is Chagas' disease. There are at least 20 million infected people[1]. The causative agent of this disease is *Trypanosoma cruzi*, which presents a biological cycle that includes various forms: epimastigotes and trypomastigotes (with flagellum) and amastigotes (without flagellum) which is the intracellular form in the vertebrate host[2].

The tricyclic antidepressant and anticalmodulin drugs such as clomipramine and trifluoperazine have trypanocidal effect[3, 4] not only on the bloodstream forms of *T. cruzi*, as reported by Hammond et al.[5], but also upon the vitality of epimastigotes. Besides, when these drugs were used for treatment of *T. cruzi* infected mice, parasitemias were reduced or disappeared.

The present experiments were carried out in order to investigate the effect of promethazine (PRO) on *T. cruzi;* this drug is another phenothiazine which is also an amphiphilic, cationic tricyclic drug and exerts anticalmodulin actions[6]. The following are the results of in vitro and in vivo studies.

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Materials and Methods

Chemicals

Promethazine hidrochloride was obtained from Sigma Chemical Co. St. Louis, MO, USA. All other chemicals were analytical reagents of the highest purity available.

Source of parasites

Epimastigotes of *T. cruzi*, Tulahuen strain, stock tul 0, were grown in a modified Warren liquid medium[7] at 28°C. Bloodstream trypomastigotes, Tulahuen strain, were obtained from infected mice; the infected heparizined blood was used for the assays described below without parasite purification.

Assays of trypanocidal effects

To test the effect of PRO on epimastigote growth, the drug was added to the culture medium in doses from 50 μM to 500 μM without changing the final medium volume.

Assays of inhibition of motility

Blood from infected mice containing bloodstream trypomastigotes was incubated at different times and in several PRO concentrations. The number of motile forms was determined by using a Neubauer hemocytometer. Parasite death was corroborated by staining with 0.1% eosin.

Determination of infectivity

Infectivity of the PRO treated trypomastigote was tested by intraperitoneal injection into 30 g Albino Swiss mice. Survival was monitored every day and parasitemia was checked weekly by microscopic observation of blood obtained by cutting the tip of the tail. Histopathological analysis of hearts were done 6 months post inoculation.

Assay of therapeutic effect

Albino Swiss male mice 30 ± 1 g were challenged with 7 x 10^4 trypomastigote forms of *T. cruzi*, Tulahuen strain, by an intraperitoneal injection. Then they were divided in 2 groups.

Group 1: starting four hours after infection, they were treated with a daily intraperioneal dose of PRO 55 mg/Kg. This dose was defined upon determination of lower and higher doses of the drug that had no effect and toxic effect, respectively.

Group 2: was injected daily with isotonic saline solution and constituted the control group.

Parasitemia of both groups was determined in Neubauer hemocytometer in blood samples obtained from the tail twice a week. Survival was monitored every day.

Electron microscopy

Ultratructural studies were performed on pellets of bloodstream trypomastigotes without treatment and pretreated with 2 µM of PRO for 5, 10 and 15 min. Pellets were fixed with Karnovsky solution for 2 h at room temperature, rinsed with cacodilate buffer 0.1 M, pH 7.35, and postfixed with 1% osmium tetroxide for 2 hours. Samples were dehydrated with acetone and included in Durcupam plastic. The ultrathin sections were mounted in a 200 mesh grid and contrasted with uranyl acetate and lead citrated. Observations were made with a Philips electron microscope.

Statistical analysis

Data were compared by analysis of variance and differences between groups were determined by Student's test with a significance level of 0.05.

Results

Figure 1 shows that, when 200 µM of PRO was added to the culture medium, the growth of epimastigotes was completely inhibited; lower doses produced lower effects. The doses capable of inhibiting epimastigote growth were high when compared to those capable of abolishing trypomastigote motility.

Table 1 shows that a concentration of 2 µM PRO was sufficient to arrest the trypomastigote motility in 5 minutes. Furthermore, a dose dependent effect was observed at lower concentrations. Parasites treated with 2 µM of PRO, appeared dead when stained with 0.1% eosin.

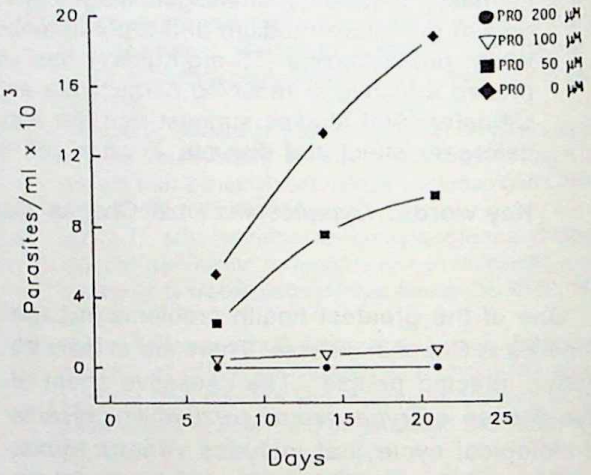

Fig. 1.— Effects of different concentrations of promethazine on the growth of *T. cruzi* epimastigotes in culture.

TABLE 1.— *Effects of promethazine on the motility (expressed as % of motile forms) of trypomastigotes of* Trypanosoma cruzi *obtained from blood samples of infected mice*

	Promethazine (µM)				
Tiempo	0	0.1	0.5	1	2
5 min	100	80 ± 5	31.20 ± 4.3	1.69 ± 0.1	0
15 min	100	46 ± 5	25.96 ± 0.4	0	0

Determinations were carried out 5 and 15 min after drug addition. The number of experiments was 20 in each group. Results are expressed as Mean ± SE.

In order to investigate infectivity of drug-treated trypomastigotes, they were injected intraperitoneally into susceptible mice. No parasites were detected in blood samples along the 6 months during which parasitemia was verified weekly. The survival of these mice was similar to that of the healthy animals, and the histopatho-logical analysis of the heart carried out 6 months post inoculation demonstrated a cardiac structure not different from non-infected mice.

The ultrastructure studies of trypomastigotes incubated for 5, 10 and 15 min with 2 µM of PRO, showed morphological alterations. Figure 2 shows a non-treated parasite (control). PRO progressively induced parasite membrane alterations until a total cell membrane disruption (Figs. 3a, 3b, 3c).

In Figs. 2a and 2b membranous lamellar structures can be observed in the cytoplasm. These structures appeared only in the PRO treated trypomastigotes. Mitochondria (Fig. 3a insert) and flagellum (Fig. 3b) presented a morphologically normal ultrastructure. Fig. 3b also shows the beginning of the cell membrane disruption and Fig. 3c, after 15 min of incubation, severe structural alterations with cell membrane disruption and cytoplasm organoids free in the extracellular space.

The in vivo assays were carried out using *T. cruzi* infected mice which were divided into two groups: group 1 was treated daily intraperitoneally with PRO (55 mg/Kg) and group 2 consisted of non treated animals (control). Figure 3 shows that in non-treated mice parasitemia rose up to 6×10^5 parasites/ml and all animals died about 12 days post infection. In group 1, PRO treatment induced a significant parasitemia reduction and survival increased from 12 to 25 ± 5 days. At death, all parsitemia values were significantly lower than in controls ($p < 0.01$).

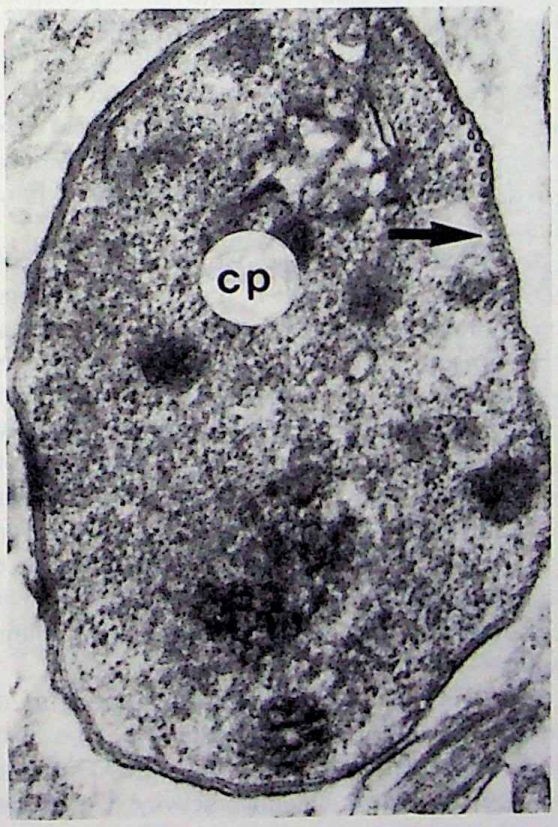

Fig. 2.— (CP) Bloodstream trypomastigotes of *T. cruzi* (control) The arrow shows subpellicular microtubules. Original magnification = X 11500

Discussion

In agreement with Hammond et al.[5], our results demonstrate that PRO has lethal effects on trypomastigotes of *T. cruzi* in vitro. Based on the loss of motility we presume that the drug began to be effective at a concentration of 0.5 µM reaching its maximum effect at 2 µM, 5 minutes after treatment. Moreover, it was also effective against epimastigotes.

Even though small doses, related to those used in human beings, were enough to render trypomastigotes completely ineffective, a concentration of 200 µM was needed to prevent epimastigote growth in culture. This dose is high when compared to 50 µM trifluoperazine which produced the same effect[4].

It has been reported that *T. cruzi* has a complex profile of calcium-dependent calmodulin binding proteins[8-12]. It is interesting to take into account that epimastigote and amastigote stages have the largest number of calmodulin binding proteins, whereas trypomastigotes only have a small content of these proteins[13].

Keeping in mind that PRO has anticalmodulin properties and that in our experiments the drug was more effective against trypomastigotes, mechanisms of inhibition of *T. cruzi* calmodulin could be associated with the trypanocidal effect of this agent. This hypothesis could be in agreement

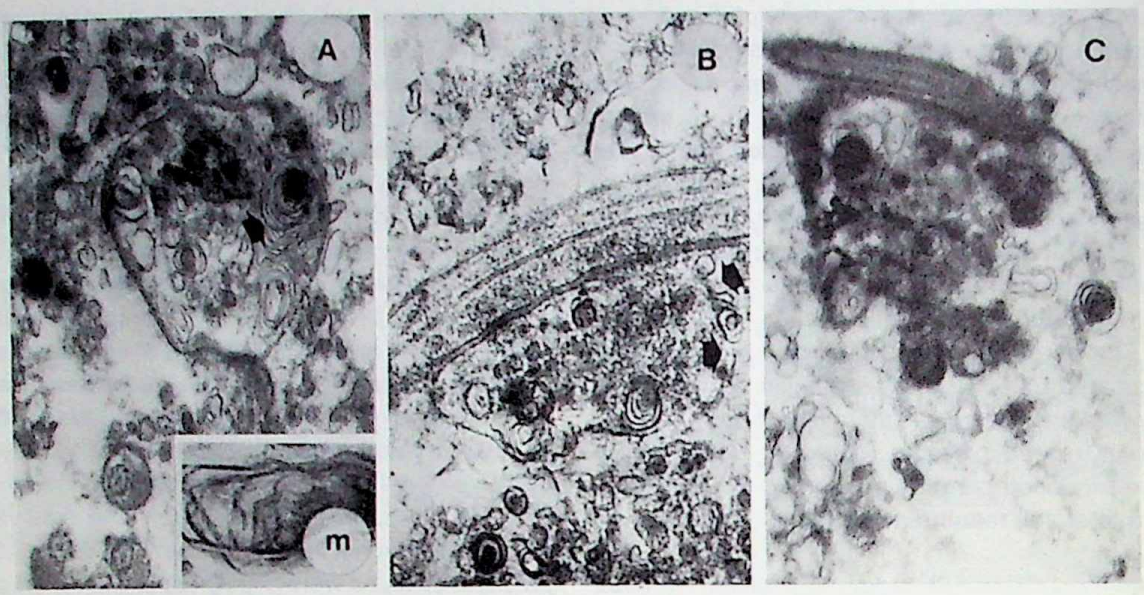

Fig. 3.— Bloodstream trypomastigotes of *T. cruzi* treated with promethazine, for 5, 10 and 15 min.
a) Lamellar structures (short arrows) inside a parasite. (m) mitochondria (insert). Original magnification = X 11500. 5 min of incubation.
b) Arrows show the beginning of cell membrane disruption. Original magnification = 11500. 10 min of incubation.
c) Severe ultrastructure alteration in the whole parasite with disruption of cell membrane. Original magnification X 11500. 15 min of incubation.

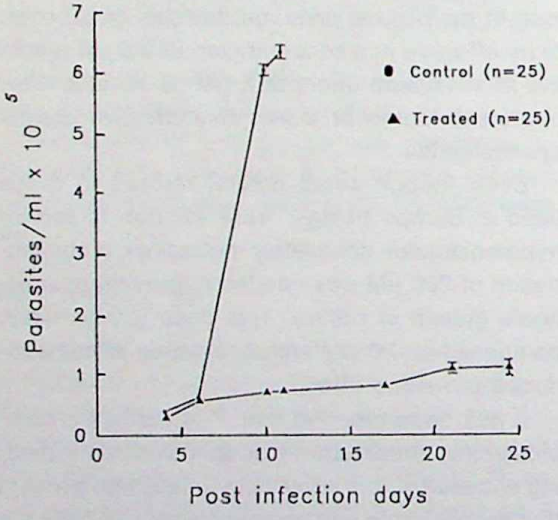

Fig. 4.— Parasitemia evolution in non-treated and treated with 55 mg/Kg/day promethazine; n = number of experiments.

with the electron microscopic studies described herein. They show that PRO induced disruption of cell membrane of *T. cruzi* thus allowing the content of the cytoplasm to be freed into the extracellular medium. This membrane alteration could be due to a detergent effect of the drug, which would also be responsible for the lamellar structure that appeared in the PRO-treated parasites.

These lamellar bodies, which were also described by Castro et al.[14] in host cells pretreated with phenothiazines, seem to be a redistribution of membrane lipids after the disruption produced by PRO. Mitochondria appeared conserved, differently from the trifluoperazine action[4] which induced mitochondrial disruption, even though both drugs are quite similar in their effects and in their chemical structure. Flagellar microtubules were also conserved upon treatment with either of these drugs, which have anticalmodulin activity that putatively prevents microtubule depolimerization[15, 16].

In summary, PRO is currently used for its antipsycotic and ansiolitic effects and is not toxic for human host at therapeutic dosis. This phenothiazine with anticalmodulin activity[6, 17] displayed trypanocidal effect upon trypomastigotes, at therapeutic concentration, and on epimastigotes of *T. cruzi*.

The in vivo experiments demonstrate that PRO affected parasitemias and significantly increased

survival of treated mice, even though the doses needed were high when extrapolated for the human host. The present results show that the lethal effects of PRO on *T. cruzi* could be attributed to severe actions on parasite membrane.

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Resumen

Efectos tripanocidas de prometazina

Prometazina es corrientemente usada por sus efectos antipsicóticos y ansiolíticos. Es una fenotiazina, no tóxica para el ser humano a dosis terapeúticas, con marcada acción anticalmodulina. Los presentes resultados muestran que prometazina tuvo efecto tripanocida sobre epimastigotes y tripomastigotes de *T. cruzi*; 200 µM inhibieron el crecimiento de epimastigotes en cultivo y 2 µM fue una concentración letal para tripomastigotes. Se utilizó también la prometazina para el tratamiento de ratones infectados con 70.000 parásitos/animal. La reducción de parasitemias y el incremento significativo de la sobrevida se logró con una dosis de 55 mg/Kg/día. Los estudios ultraestructurales demostraron que los efectos letales de esta fenotiazina están relacionados con el efecto detergente que provoca sobre la membrana celular del *T. cruzi*.

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