Effects of Juvenile Hormone Analogues (JHA) on the Development of *Trypanosoma cruzi*

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Juvenile hormone analogues were tested for their lytic activity on *Trypanosoma cruzi* Chagas, 1909 blood tripomastigotes cultivated *in vitro*. The results indicated that the carbamate **4** and the phenoxyphenol derivative **1** are considered good candidates for blood sterilization.The compounds were also assayed for inhibition of development of parasites in infected mice showing a moderated degree of activity.

Previous results have indicated that juvenile hormone analogues (JHA) showed effects on the development of eggs and nymphal stages of triatomine Chagas' disease vectors, and biological activity on *Trypanosoma cruzi* Chagas, 1909 epimastigotes (Rodriguez *et al.*, 1988; Rodriguez *et al.*, 1989; Stoka *et al.*, 1990; Rodriguez *et al.*, 1991). It has been also shown that the insect *Triatoma infestans* Klug, 1834, Chagas' disease vector, after treatment with JHA is less susceptible to gut infections with *T. cruzi* than normal non-treated insects (Perlawagora-Szumlewicz *et al.*, 1975).

Those results encouraged the search for more active compounds against *T. cruzi*, and we wish to inform the preparation of additional JHA and their *in vitro* lytic activities on the microorganism causing Chagas' disease. Besides, some of the new compounds were tested in *in vivo* experiments on infected mice.

Materials and methods

Assays

In vitro. Trypanosoma cruzi infected mice blood was used to test the action of different concentrations of JHA. The incubations were performed in polystirene 96 well U-plates at 4 °C during 24 h. In all experiments 100 µl of blood containing different quantities of trypomastigotes per ml and 2 µl of ethanolic solution of JHA were used. Ethanol (2 µl) was used as control.

In vivo. Male 60-day-old BALB/c mice received 5 daily doses of different JHA in ethanol by the oral route (90 µg of JHA/mouse/day), 30 min after infection of mice with 500 blood trypomastigotes via i.p.

Juvenile hormone analogues. The assayed compounds are shown in Fig. 1. Their respective preparations have been described in literature; **1**: 4-phenoxyphenyl prop-2-en-1-yl ether (Rodriguez *et al.*, 1991); **2**: 4-phenoxyphenoxyethyl tetrahydropyranyl ether (Rodriguez *et al.*, 1989); **3**: S-ethyl (3,7,11-trimethyl-10(*R,S*),11-epoxy-dodeca-2*E*,6*E*-dien-1-yl)-thiolcarbonate (Rodriguez *et al.*, 1991); **4**: 2-{4-[2-(ethoxycarbamato)-ethoxy]-benzyl}-1-cyclohexanone ethylene acetal (Wimmer *et al.*, 1985).

Results

The results on the lysis of *T. cruzi* (trypomastigotes) by different concentration of the JHA are shown in Table I while the results obtained in the *in vivo* experiments are summarized in Table II.

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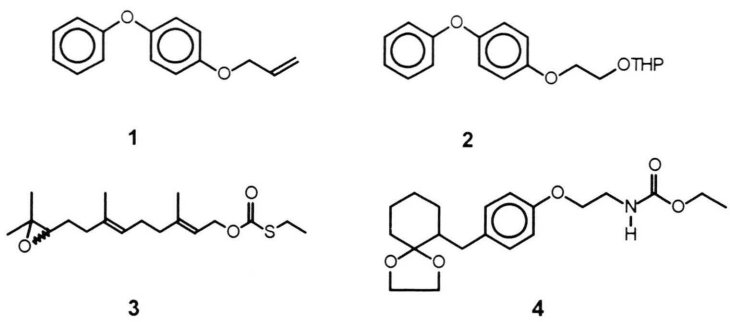

Fig. 1. Juvenile hormone analogues assayed as inhibitors of *Trypanosoma cruzi* replication.

Table I. Lysis of *Trypanosoma cruzi* blood trypomastigotes by treatment with juvenile hormone analogues at 4 °C.

Compound	Parasites [ml×10^5]	Concentration [μmol/l]	% Lysis [X ± SD]	Number of experiments
1	5	200	65.5 ± 18.1	3
1	5	500	76.4 ± 12.4	3
1	5	1000	94.0 ± 1.4	3
1	10	200	69.8 ± 11.9	5
1	10	500	77.1 ± 9.7	10
1	10	750	83.3 ± 7.8	3
1	10	1000	88.6 ± 7.3	7
1	50	100	59.0 ± 2.0	2
1	50	200	70.3 ± 2.0	3
1	50	500	78.0 ± 9.9	3
1	50	1000	87.3 ± 10.5	4
2	5	200	59.5 ± 8.9	3
2	5	500	79.1 ± 4.4	2
2	5	1000	83.1 ± 10.3	2
2	10	200	66.2 ± 17.2	6
2	10	500	73.8 ± 9.9	9
2	10	750	81.5 ± 4.5	2
2	10	1000	83.2 ± 8.2	10
3	5	500	61.8 ± 16.7	4
3	5	1000	80.2 ± 10.9	4
4	5	200	53.5 ± 14.3	3
4	5	500	95.0 ± 5.0	6
4	5	750	97.1 ± 4.5	4
4	5	1000	100.0 ± 0.6	6

Table II. Effect of compounds **1, 2, 3** and **4** on the parasitemia of mice infected with *Trypanosoma cruzi*[a].

Blood trypomastigotes	JHA[b]	Parasitemia [X ± SD]	Survival [%]
500	1	1813 ± 1529	12/17 (71)
500	2	2293 ± 1457	7/11 (64)
500	3	1881 ± 1236	5/10 (50)
500	4[c]	1570 ± 717	2/8 (25)
500	ethanol[d]	3151 ± 2149	5/14 (36)

[a] Average of 2 experiments.
[b] 5 daily doses, 90 μg/mouse/day, 10 μl.
[c] Daily doses, 180 μg/mouse/day, 10 μl.
[d] 5 daily doses, 10 μl.

Discussion

The general aim of this project is to control the development of *Trypanosoma cruzi* in insects as well as in mammals. Despite of the progresses made in chemotherapy, new compounds are needed because the trypanocidal drugs presently in use, Nifurtimox and Benzmidazole, cause considerable side effects on patients. There is no effective treatment available for Chagas' disease in spite of the important progresses made in the study of the biochemistry of the microorganism responsable for the mentioned disease (Marr and Docampo, 1986; Morello, 1988; Gutteridge, 1985).

Bearing in mind the different studies on the action of host's hormones on parasites, and our preliminary experiments in which we have observed that JHA produce an inhibition of growth of *T. cruzi* epimastigotes *in vitro*, we have hypothesized the probable action of JHA in the different stages of *T. cruzi*. Moreover, and due to the risk that *T. cruzi* may be transmitted in blood bank for transfusions, it is very important to have new compounds to kill parasites in blood to be transfused. At present, Gentian Violet is in use for this purpose but it suffers of serious limitations concerning its safety (Gutteridge, 1985). Accordingly, several JHA were tested in *in vitro* experiments performed on blood from mice infected with *T. cruzi* trypomastigotes. When parasites were expose to the synthetic products (see Table I) compounds **1** and **4** showed the higher lytic effect on mouse blood trypomastigotes. Compound **4** at 1000 μmol/l lysed 100% of trypomastigotes when incubated with 5×10^5 parasites/ml. In similar conditions, the best lytic effect of compound **1** was 94%. The values determined for compounds **2** and **3** indicated that their lytic capacity, although

similar between them, are inferior to those obtained with **1** and **4**. The respective values registered for these compounds indicated that they could be used for blood sterilization.

In experiments *in vivo*, JHA showed a moderated degree of activity against the parasites. As seen in Table II, the best percentages of survival were observed in mice treated with compounds **1** and **2** (71 and 64% respectively) compared with control mice (36%). Unexpectedly, compound **4** did not present activity in these *in vivo* assays even when tested at twofold doses than those used for the other compounds.

Although the differences were not statistically significant, parasitemia level of mice treated with **1** and **2** were lower than those from control mice.

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