

## ULTRASTRUCTURAL ALTERATIONS OF INTRACELLULAR STAGES AND EFFECTS ON BLOOD FORMS OF *TRYPANOSOMA CRUZI* INDUCED *IN VIVO* BY 2-AMINO-5-(1-METHYL-5-NITRO-2-IMIDAZOLYL)-1,3,4-THIADIAZOLE<sup>1</sup>

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*An electron microscopy study shows that the administration of a single dose (500 mg/kg, p.o.) of 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1, 3, 4-thiadiazole induces in mice infected with Trypanosoma cruzi results in degenerative lesions of the intracellular stages. Ultrastructural alterations are detected as early as 6 hours after the drug administration and destruction of the parasites occurs within 18 – 36 hours. Trypomastigotes are cleared from the bloodstream 4 to 6 hours after treatment. The combined effect on both developmental stages is apparently responsible for the in vivo effects of this drug which is the most active drug ever tested in our laboratory in experimental Chagas' disease.*

Key words: *Trypanosoma cruzi*. Nitroimidazo-thiadiazole. Electron microscopy.

Recent investigation has demonstrated a remarkable curative effect of 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1, 3, 4-thiadiazole in mice experimentally inoculated with *Trypanosoma cruzi*<sup>6</sup>. The drug has been further investigated *in vivo* and we now report the fine structure alterations of *T. cruzi* intracellular stages in treated animals as well as an intense suppressive action on circulating blood forms induced by the compound. The early and intense activity of this drug on both parasite stages explains its unusual high curative effect in experimental Chagas' disease.

### MATERIALS AND METHODS

*Ultrastructural studies.* Male albino mice weighing 18 – 20g were intraperitoneally inoculated with  $5 \times 10^4$  blood trypomastigotes of the *Colombiana* strain<sup>1</sup>. 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1, 3, 4-thiadiazole (CL 64'855, "Megazol") was given by oral route in a single dose of 500 mg/kg, 12 days after inoculation. Untreated mice inoculated with the same inoculum were used as controls. Both treated and control animals were sacrificed 6, 12, 18, 24 and 36 hours

after treatment. The heart was removed and fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer. The organ was sectioned at the level of the atrio-ventricular septum; the atria were then kept for 12 hours at 4°C in the glutaraldehyde solution, post-fixed with 1% osmium tetroxide in 0.1M phosphate buffer for 3 hours, dehydrated in alcohol series and, finally, embedded in Epon. Ultrathin sections were obtained in a MT2 Porter Blum ultra-microtome, stained in uranyl acetate and Reynold's stain, and examined in a Zeiss EM-10 electron microscope.

*Effect on bloodstream forms.* The technique described by Filardi & Brener<sup>7</sup> was used, as follows: male albino, weighing 18 – 20g, were inoculated with  $1 \times 10^5$  bloodstream forms of strains *Colombiana*<sup>1</sup>, VL-10<sup>11</sup> and CL<sup>3</sup>. At the peak of parasitemia (which was at days 9 – 11 with CL strain and 15 – 20 with *Colombiana* and VL-10 strains) the animals received a single dose of 500 mg/kg of the drug by oral route. A group of inoculated but untreated animals was used as control. The parasitemia was determined according to Brener<sup>2</sup> before and 6 hours after drug administration. The reduction in the number of parasites was estimated by comparing the number of parasites after treatment with that detected before drug administration.

### RESULTS

A remarkable finding of the ultrastructural study was the occurrence of extremely early alterations of *T. cruzi* intracellular stages. Whereas in the controls the parasites displayed normal morphology

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(Fig. 1) a number of ultrastructural alterations were observed as soon as 6 hours after drug administration in the treated animals. The initial lesions were vacuolization of the cytoplasm followed by swelling of the nuclear envelope, rough endoplasmic reticulum and Golgi cisterns (Fig. 2); a similar swelling was detected in the area around the fibrils network of the kinetoplast. These ultrastructural changes are progressively more severe in the animals sacrificed at the further periods of examination after treatment, showing a clear reduction of the number of ribosomes and other cytoplasmic components after 18 hours (Fig. 3). Even in those degenerated parasites some structures remain apparently intact such as the sub-pellicular microtubules, plasmatic membrane and the kinetoplast fibril network. At 36 hours degenerated forms displaying partial or total lysis are highly predominant (Fig. 4). In the untreated control group the great majority of the intracellular stages remained morphological normal.

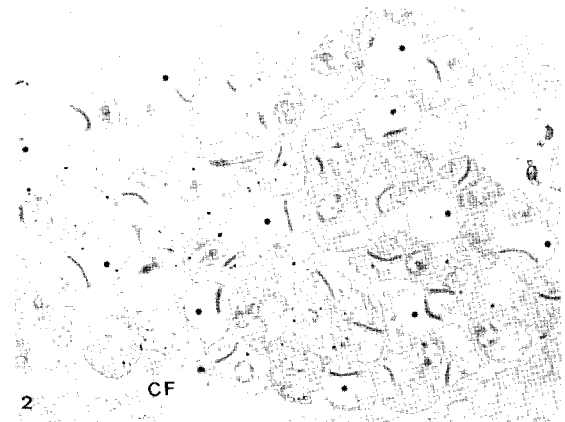


Fig. 2 - Treated, 6 hours. *T. cruzi* intracellular amastigotes showing early vacuolization of the cisterns of the endoplasmic reticulum, nuclear envelope and kinetoplast. X 5,700.

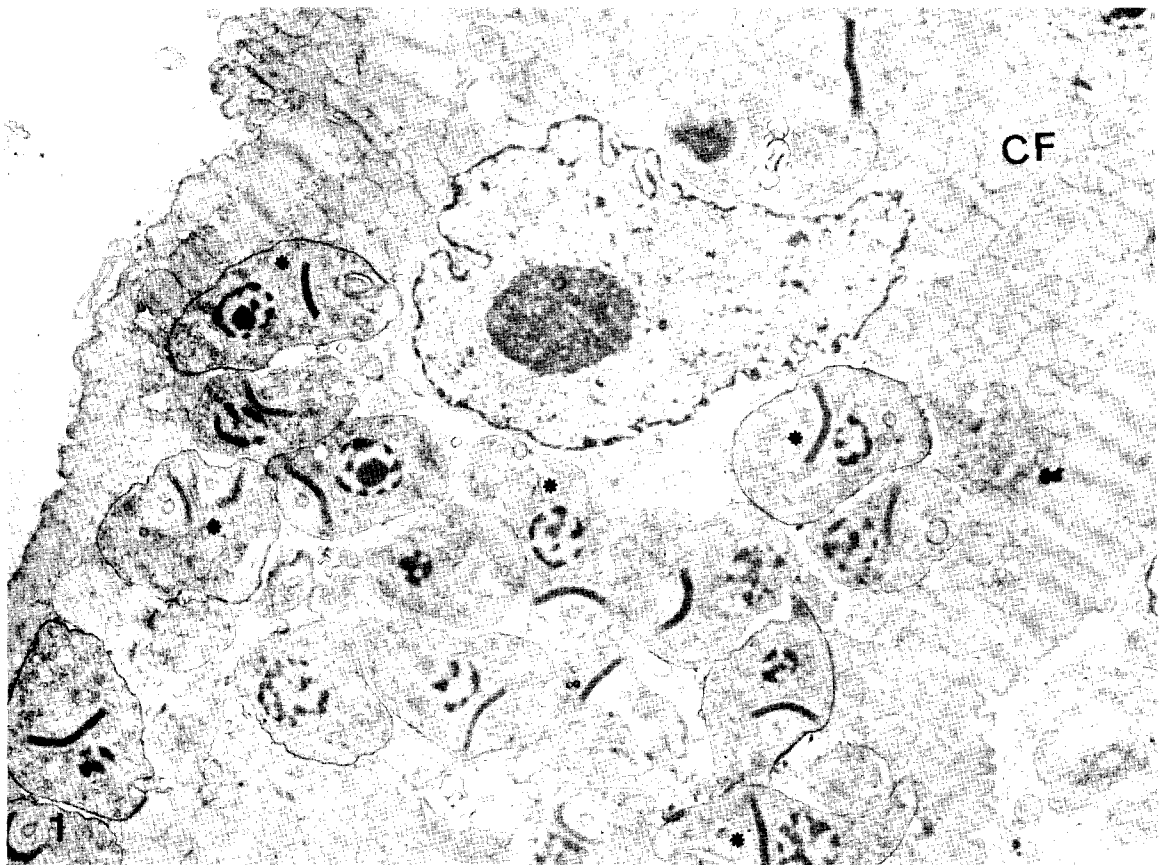


Fig. 1 - Control. *T. cruzi* intracellular amastigotes (asterisk) displaying normal morphology inside the cardiac fibre (CF). X 7,300.

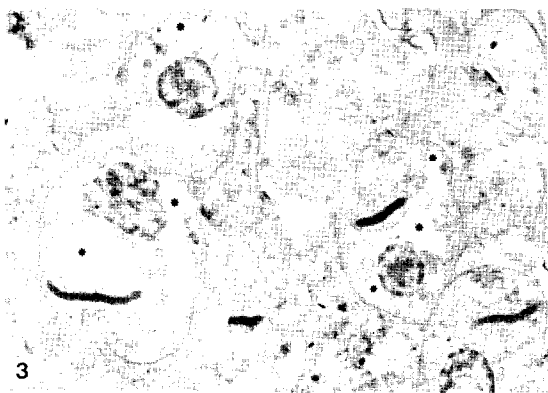


Fig 3 - Treated, 18 hours. *T. cruzi* intracellular amastigotes with progressive lesions of the membranous structures of the endoplasmic reticulum and nuclear envelope; and rarefaction of the cytoplasmic components. X 13,000.

Fig. 5 shows the effects of CL 64'855 on the parasitemia in mice inoculated with *T. cruzi* strains VL-10, Colombiana and CL. A marked and rapid

decline in the number of circulating trypomastigotes was observed as soon as 6 hours after drug administration, showing that the early effects on blood forms parallel that described for the intracellular stages.



Fig 4 - Treated, 36 hours. *T. cruzi* degenerated amastigotes. Lysis of membranes and cytoplasmic components. X 10,700.

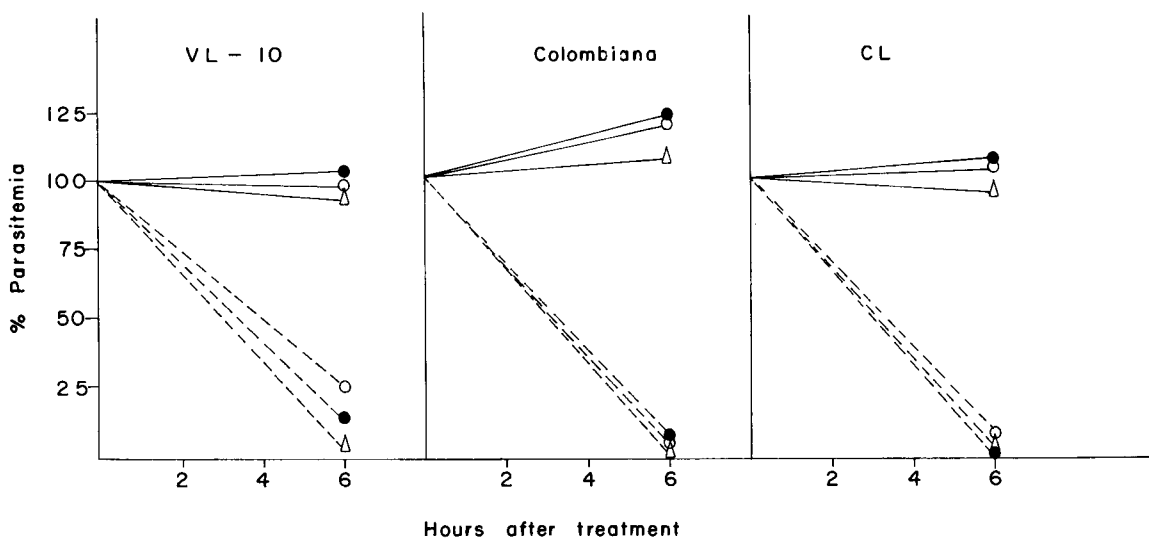


Fig. 5 - Reduction of parasitemia induced by a single dose (500 mg/kg, oral route) of CL 64'855 in mice inoculated with strains VL-10, Colombiana and CL of *T. cruzi*. Experiments performed with groups of three mice. Animal controls are represented by solid lines (—) and treated by dashed lines (---).

## DISCUSSION

The compound 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1, 3, 4-thiadiazole is apparently the most active drug so far tested against *T. cruzi* experimental infections<sup>6</sup>. According to our data it cures 85 – 100% of the inoculated animals treated for 20 days with doses of 50 – 100 mg/kg; single doses of 500 mg/kg cure 88.8% of mice inoculated with the *T. cruzi* Y strain. Moreover, it also cures 85 – 100% of the animals inoculated with strains Colombiana and VL-10 which are highly resistant to nitrofurans and 2-nitroimidazole derivatives<sup>11</sup>. In this paper we describe the effects of this drug on both intracellular and circulating *T. cruzi* stages. The early and marked effects of single doses apparently provide an explanation for high curative rates detected after single drug administration and strongly indicate an irreversible curative action of this compound.

The pattern of the intracellular parasite destruction with compound CL 64'855 seems to be similar to that found in animals or in tissue culture treated with nitrofurazone<sup>4</sup> and nifurtimox<sup>12</sup>. Basically, they consist of an increase in the number of cytoplasmic vacuoles, reduction of the ribosomes and swelling of the organelles. The process ends in the lysis of the parasites with persistence of the plasma membrane and the kinetoplast. The molecular mechanism of the lesions induced by CL 64'855 is not yet known. Docampo et al<sup>5</sup> demonstrated that incubation of *T. cruzi* homogenates with a nitrofurans derivative ("nifurtimox") favors the appearance of a nitroaromatic anion radical which reacts with oxygen and induces an increased rate production of superoxide anion and hydrogen peroxide, which are involved in the trypanocidal effect. Benzimidazole (a nitroimidazole derivative), however, is not reduced to the nitro anion radical and does not induce generation of superoxide and hydrogen peroxide. This suggests a different (but not yet defined) mechanism of toxicity for nitroimidazole derivatives<sup>10</sup>. Since CL 64'855 is a nitroimidazole with a thiadiazole component, the mechanism of this novel compound should be specifically investigated in comparison with the nitrofurans and nitroimidazole active compounds.

The effect of drugs on blood forms has been less investigated. Haberkorn & Gönner<sup>8</sup> using a special reflex microscope observed in living blood trypomastigotes from mice treated with nifurtimox lesions such as cytoplasm vacuolization and membrane splitting. In our experiments the decline in the number of circulating trypomastigotes occurs very soon after drug

administration and parallels the early action against intracellular stages. Data from our laboratory show that macrophages play an important role in the clearance of blood parasites by removing the circulating trypomastigotes in a process which begins very soon after the drug administration<sup>9</sup>. The effect on both intracellular and circulating stages is responsible for the rapid and extremely intense activity of this compound.

## RESUMO

*Um estudo com microscopia eletrônica mostrou que a administração de dose única (500 mg/kg, p.o.) do composto 2-amino-5-(1-metil-5-nitro-2-imidazolil)-1, 3, 4-tiadiazole induz, em camundongos inoculados com T. cruzi, lesões degenerativas das formas intracelulares do parasita. Alterações ultra estruturais são observadas 6 horas após a administração da droga e destruição ocorre em 18 – 36 horas. Tripomastigotas também desaparecem do sangue circulante dos animais 4 – 6 horas após o tratamento. O efeito em ambos os estágios evolutivos do T. cruzi é aparentemente responsável pelos efeitos in vivo desse derivado que é o mais ativo composto já testado em nosso laboratório na doença de Chagas.*

Palavras chaves: *Trypanosoma cruzi*. Nitroimidazol – Tiadiazole. Microscopia eletrônica.

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