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LECTIN RECEPTORS DISTRIBUTION IN THE SURFACE MEMBRANE OF *TRYPANOSOMA CRUZI* BLOOD FORMS COLLECTED FROM MICE SUBMITTED TO SPECIFIC TREATMENT

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The authors investigated the distribution of lectin receptors on Trypanosoma cruzi ***blood forms collected from mice inoculated with, respectively, the drug-resistant and drug-sensitive strains VL-10 and CL, and treated with the two standard active nitroheterocyclic compounds nifurtimox and benznidazole used for treatment of human Chagas' disease. Blood trypomastigotes purified in Fycoll-Hypaque were incubated with fluorescein-labelled lectins Con A, WGA, EE, WFA, TPA and PNA and then microscopically examined. Neither qualitative or quantitative differences in the fluorescence intensity could be detected between the parasites from VL-10 and CL strains submitted or not to treatment. The results suggest that both strains do not differ in their surface membrane carbohydrate moieties. Moreover, the rapid clearance of blood forms from the drug-sensitive strain in animals treated with single doses of both compounds is not likely to depend on membrane alterations expressed by changes in the carbohydrate components. Furthermore, resistance or sensitivity to drugs is not apparently related to carbohydrate distribution on*** T. cruzi ***blood forms.***

Key words: ***Trypanosoma cruzi*** - treatment - lectin receptors

Single doses of nitroheterocyclic derivatives active against ***Trypanosoma cruzi*** induce in mice, within 4-6 h, a marked decrease in the number of the parasite blood forms (BTry) (Filardi & Brener, 1984). The mechanism of this rapid parasite clearance is not yet known but Lages-Silva et al. (1990) have recently demonstrated that the ***in vitro*** phagocytosis by mouse peritoneal macrophages of BTry collected from mice treated with single doses of these active compounds is significantly enhanced as compared with parasites obtained from untreated animals. Such results suggest that this rapid disappearance of BTry caused by macrophages or other mechanism might depend on previous alterations of the parasite surface membrane. In the present study we have investigated, using lectins of different specificities, the existence of quantitative and/or qualitative differences in the surface membrane carbohydrate moyeties from BTry of ***T. cruzi*** resistant and sensitive strains collected from animals submitted to specific treatment.

MATERIALS AND METHODS

Trypanosoma cruzi ***strains*** — CL, isolated from a naturally infected ***Triatoma infestans*** collected in Rio Grande do Sul, Brazil (Brener & Chiari, 1963) and VL-10, isolated from a chronic chagasic patient living in Virgem da Lapa, MG, Brazil (Schlemper Jr et al., 1983). The strains CL and VL-10 have been demonstrated in animals to be respectively sensitive and resistant to the standard drugs benznidazole and nifurtimox used clinically in Chagas' disease (Filardi & Brener, 1987).

Drugs used — 3-methyl-4(5'-nitrofurfurylidene-amino)-tetrahydro-4H-1,4-thiazine-1,1-dioxide (nifurtimox) and N-benzyl-2-nitro-1-imidazolacetamide (benznidazole).

Lectins and specific carbohydrates — The following fluorescein-labelled lectins were used: Peanut Agglutinin (PNA), Concanavalin A (Con A), ***Tetragonolobus purpureas*** (TPA), Wheat Germ Agglutinin (WGA), ***Wisteria floribunda*** (WFA) and ***Euonymus europaeus*** (EE) with specificity for, respectively, the carbohydrates D (+) galactose, α-methyl-D-mannoside, L (−) fucose, N-acetyl-D-glucosamine, N-acetyl-D-galac-

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tosamine and D-galactosyl (1,3)-D-galactose. In some experiments non-fluoresceinated Con A was used in agglutination tests. All reagents have been purchased from Sigma Chemical Co, St. Louis, Mo., USA.

Mice treatment — The rapid test described by Filardi & Brener (1987) to evaluate the susceptibility of *T. cruzi* BTry to drugs was used. Briefly, male albino mice were inoculated i.p. with 10^5 *T. cruzi* BTry from CL or VL-10 strains and treated at the peak of parasitemia with 125 mg/kg of either benznidazole or nifurtimox. The parasitemia was determined according to Brener (1962) just before drug administration and 2 h after, when the parasites were collected. In order to assess the suppressive effect of the drugs, treated and untreated controls had their parasitemia also determined 6 h after treatment.

Direct fluorescence test (DF) — Blood from treated and untreated animals was collected by bleeding animals through the retro-orbital sinus and defibrinated with glass beads. BTry were isolated and purified by centrifugation in a Ficoll-Hypaque gradient (Budzko & Kierszenbaum, 1974), washed with medium 199 plus 10% fetal calf serum, diluted at 5 x 10^6/ml and air fixed in glass slides overnight at room temperature. The fixed BTry were incubated with different lectin concentrations (5,000-500 to 0.12 μg/ml) for 30 min at room temperature, repeatedly washed with PBS pH 7.2, air dried, covered with glycerol and examined in an Ortholux Wild-Leitz microscope equipped with a HBO 500w CAC lamp. Specificity binding assays were carried out by incubating for 30 min the lectin in the minimum concentration which induced direct fluorescence with different concentrations of the specific carbohydrate and then performing the direct test as above described.

Direct agglutination test — Twenty-five μl of Con A at different concentrations (1,500 to 6 μg/ml) and 25 μl of a living BTry suspension (7 x 10^7 /ml) were deposited in microtitration plate wells, left for 1 h at room temperature and then microscopically examined after 30, 60 and 120 min. Specificity binding tests were performed as described above using the specific carbohydrate α-methyl-D-mannoside.

RESULTS

Figures 1 and 2 show the percentages of parasitemia reduction in mice inoculated, respectively, with CL and VL-10 *T. cruzi* strains, treated with nifurtimox and benznidazole. The reduction of parasitemia after 6 h treatment induced by nifurtimox and benznidazole, was, respectively, 93% and 97% for the CL strain, 10% and 8% for VL-10. No significant differences in the fluorescence induced by the various lectins could be detected between parasites from both strains collected from animals treated either with benznidazole or nifurtimox and untreated controls. The minimum lectin concentrations (μg/ml) inducing direct fluorescence in *T. cruzi* CL and VL-10 strains BTry collected from mice treated with single dose of either benznidazole or nifurtimox (125 mg/kg) and from untreated controls were Con A: 0.5, WGA: 1.0, WFA: 2.0, EE: 6.0, TPA: 16.0 and PNA: 40.0. Binding of Con A, WGA, WFA, EE, TPA and PNA to the parasites was inhibited by their specific sugars at the concentrations of,

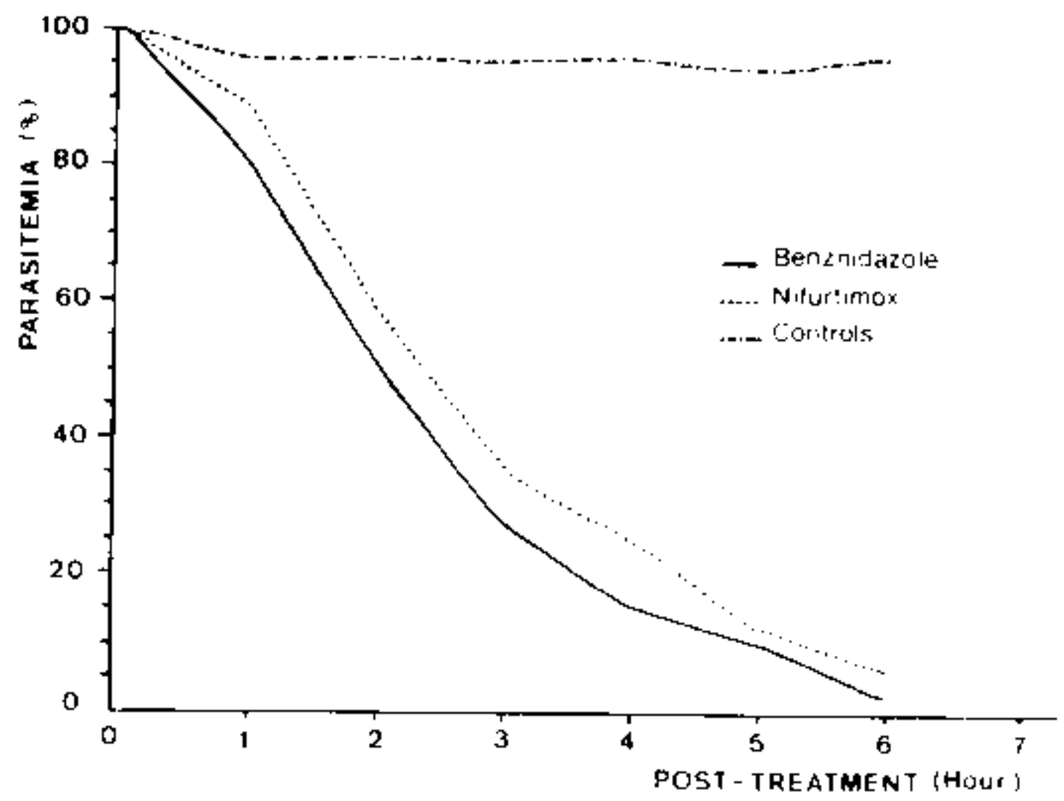

Fig. 1: percentage of parasitemia reduction in mice inoculated with *Trypanosoma cruzi* CL strain and treated with a single oral dose of benznidazole and nifurtimox (500 mg/kg).

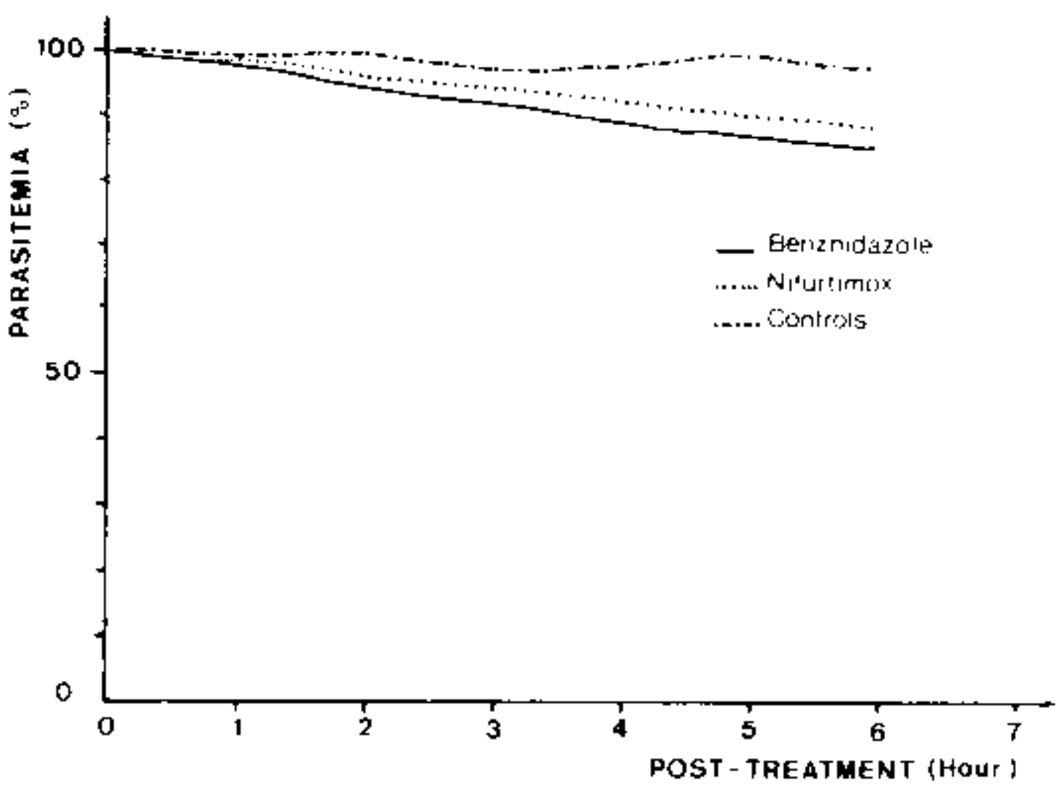

Fig. 2: percentage of parasitemia reduction in mice inoculated with *Trypanosoma cruzi* VL-10 strain and treated with a single oral dose of benznidazole and nifurtimox (500 mg/kg).

respectively, 6.2 mM for α-methyl mannoside, 1.0 M for N-acetyl-D-glucosamine, 0.5 mM for N-acetyl-D-galactosamine, 20 mM of D-galactosyl (1-3)-D-galactose, 0.4 M of L (−) fucose and 2.0 M of D (+) galactose. Differences in the fluorescence intensity among the various lectins used were observed and are likely to express variations in the number of receptors in the BTry. Agglutination with non-labelled Con A was observed at the minimum concentration of 24 μg/ml with BTry from both strains from treated and untreated animals. At the concentration of 375 μg/ml Con A displayed, in addition to agglutination, a lethal effect on both parasite populations.

DISCUSSION

Studies carried out with lectins have identified differences in *T. cruzi* strains and developmental stages carbohydrate moieties (Schlemper & Schottelius, 1979; Araújo et al., 1980; Pereira et al., 1980; Muhlpford et al., 1984). On the other hand, qualitative differences have been detected by lectins in membrane surface carbohydrates between two strains of *Trichomonas vaginalis*, respectively resistant and susceptible to metronidazol (Dias Filho et al., 1988). In our experiments no evidence was provided indicating differences in the distribution of lectin receptors between CL and VL-10 *T. cruzi* strains, respectively sensitive and resistant to nifurtimox and benznidazole, the standard drugs used in human Chagas' disease. Moreover, the distribution of carbohydrates detectable by the used lectins was not either quantitatively or qualitatively influenced by the administration of both active drugs.

The data herein presented apparently do not suport the suggestion that the clearance of BTry following drug administration is related to an enhancement of the parasite phagocytosis as a result of alterations in surface membrane carbohydrates. Although using a set of lectins specific for some of the most common cell surface carbohydrates no changes in their distribution could be detected. The possibilities exist however that drugs would have affected BTry components others than lectin binding sites. In this respect Brindley & Sher (1987) demonstrated that in mice infected with *Schistosoma mansoni* the treatment with praziquantel exposes adult worms surface antigens which otherwise are not recognized by anti-schistosome antibodies. Other mechanisms of cooperation between host immune response and drug activity should be investigated in Chagas' disease.

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