EFFICACY OF CHEMOTHERAPY WITH BENZNIDAZOLE IN CHILDREN IN THE INDETERMINATE PHASE OF CHAGAS' DISEASE

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Abstract. A double-blind, randomized, clinical field trial was designed to test the efficacy and tolerance of a specific drug treatment in children in the indeterminate phase of infection by *Trypanosoma cruzi.* Children were treated with benznidazole at a dose of 5 mg/kg/day for 60 days or placebo and followed-up for 48 months. The treated children showed a significant decrease in geometric mean titers of antibodies against *T. cruzi* measured by indirect hemagglutination, indirect immunofluorescence, and ELISA. After a four year follow-up, 62% of the benznidazole-treated children and no placebo-treated child were seronegative for *T. cruzi* when tested by an ELISA using a *T. cruzi* flagellar calcium-binding protein (F29). Xenodiagnosis carried out after 48 months of follow-up was positive in 4.7% of the benznidazole-treated children and in 51.2% of the placebo-treated children. These results show the tolerance to and efficacy of benznidazole against *T. cruzi* in seropositive children six to 12 years of age. We used an early serologic marker of cure after treatment, consisting of a recombinant antigen implemented in a rapid, conventional serologic procedure.

Chagas' disease exists only in the American continents, and extends from Mexico to Argentina and Chile. In Argentina, there are approximately 2.3 million people infected with *Trypanosoma cruzi.*[1] The disease is transmitted by triatomine bugs and also by blood transfusion and transplacentally. The course of infection includes an acute phase that lasts until six months after the infection, an indeterminate phase without symptoms, and a chronic phase in which approximately 30% of the patients present clinical evidence of heart disease or megavisceras. The etiologic treatment of Chagas' disease was started in 1930 by Salvador Mazza,[2] who used a quinoline derivative (7602; Bayer, Buenos Aires, Argentina). At present, nifurtimox and benznidazole[3] are indicated for the acute phase and for recent *T. cruzi* infections in children less than three years of age.[4] The objective of this study was to test the efficacy and tolerance of a specific drug treatment in children infected with *T. cruzi* in the indeterminate phase of Chagas' disease who lived in rural areas of Salta, Argentina.

MATERIALS AND METHODS

Experimental design. This study was a double-blind, randomized, clinical field trial carried out in the Province of Salta in northwestern Argentina. The children lived in 14 localities in an area of approximately 200 km². Sixty-six percent of the children lived in rural areas. The area has been under continuous triatomine surveillance by sanitary agents since 1982 (i.e., they undertook regular insecticidal actions against infestations). This was a condition for the selection of the study area. We assumed that the children's infection with *T. cruzi* would be more than six years old. Children were excluded from the trial for any of the following reasons: 1) presence of any chronic health condition (such as epilepsy, malnutrition, asthma, severe anemia); 2) presence of any acute infectious disease; 3) lack of consent from their parents to participate in the study; and 4) unstable residence. The children were matched in each locality by age to re-

ceive benznidazole, (Radanil®; Roche, Olivos, Argentina) (benznidazole-treated children [BTCh]) or placebo (placebo-treated children [PTCh]). Fifty-five children were allocated to the BTCh group and 51 children to the PTCh group. Benznidazole (5 mg/kg/day) was administered to each child for 60 days. The pills were administered by parents, teachers, or nurses from the health services who were specifically trained for such a task.

Informed consent was obtained twice, before screening and before recruitment into the trial. The protocol was reviewed and approved by the Ethical Committee of the Instituto Nacional de Chagas Dr. Mario Fatala Chaben.

Clinical examination. Continuous medical assistance (anamnesis, physical examinations, and electrocardiograms [ECGs]) was provided during the trial. Laboratory tests (red blood cell and leukocyte counts, hematocrit, erythrocyte sedimentation rate, bilirubin, creatinine, aspartate aminotransferase, and alanine aminotransferase levels, and urine tests) were performed at days 21 and 60 after initiation of treatment to assess drug toxicity. Side effects were considered mild if the patients showed some adverse effects but were able to complete the treatment; moderate if treatment had to be suspended because of some side effect; and severe if it was suspended and the patients needed special treatment.

Serology. Repeated serologic tests were carried out immediately before and at 3, 6, 12, 18, 24 and 48 months after the onset of treatment; these included an enzymatic immunoassay (EIA),[5] an indirect hemagglutination assay (IHA),[6] and an indirect immunofluorescence assay (IFA).[7] A new EIA (F29 EIA), using a *T. cruzi* flagellar calcium-binding protein (F29) as antigen, was used to assess parasitologic cure of patients.[8] The clone encoding the whole protein was subcloned in the pMAL-p2 expression vector and expressed in *Escherichia coli.*[8]

Xenodiagnosis. All children were tested by xenodiagnosis using two boxes with 10 *Triatoma infestans* third or fourth instar nymphs each at the end of the follow-up.[9]

Statistical analysis. The data were analyzed using Epi-

TABLE 1
Serologic follow-up of children treated with benznidazole or placebo to 48 months post-treatment in Salta, Argentina, 1991–1995*

Treatment	n	IHA				IFA				EIA			
		Mean	SD	Test		Mean	SD	Test		Mean	SD	Test	
Benznidazole													
Pretreatment	51	7.98	1.82	7 DF	1 DF	7.05	1.12	7 DF	1 DF	0.467	0.099	7 DF	1 DF
End of treatment	47	7.68	2.14		NS	6.57	1.58		NS	0.433	0.110		NS
3 months	45	7.26	2.33		NS	6.27	1.28		$P<0.01$	0.409	0.112		$P<0.01$
6 months	45	7.00	2.53		$P<0.05$	6.11	1.57		$P<0.001$	0.371	0.115		$P<0.001$
12 months	48	7.00	2.27		$P<0.05$	5.87	1.56		$P<0.001$	0.369	0.107		$P<0.001$
18 months	47	6.53	2.62		$P<0.001$	5.80	1.82		$P<0.001$	0.358	0.120		$P<0.001$
24 months	46	6.80	2.26		$P<0.01$	5.32	2.03		$P<0.001$	0.330	0.098		$P<0.001$
48 months	44	5.93	2.11	$P<0.001$	$P<0.001$	5.65	2.18	$P<0.001$	$P<0.001$	0.343	0.094	$P<0.001$	$P<0.001$
Placebo													
Pretreatment	50	8.00	1.16	7 DF	1 DF	6.80	1.22	7 DF	1 DF	0.472	0.095	7 DF	1 DF
End of treatment	45	8.11	1.21		NS	6.80	1.07		NS	0.492	0.090		NS
3 months	44	8.11	1.10		NS	6.54	1.15		NS	0.489	0.098		NS
6 months	39	7.87	1.34		NS	6.61	1.60		NS	0.477	0.101		NS
12 months	47	8.08	1.26		NS	6.40	1.13		NS	0.476	0.113		NS
18 months	48	7.93	1.17		NS	6.47	1.16		NS	0.464	0.108		NS
24 months	49	7.77	1.22		NS	6.34	1.54		NS	0.479	0.104		NS
48 months	44	7.47	0.95	NS	$P<0.05$	6.97	2.21	$P<0.05$	$P<0.05$	0.501	0.115	NS	NS

* IHA = indirect hemagglutination assay; IFA = indirect immunofluorescence assay; EIA = enzyme immunoassay; Test = analysis of variance or Kruskal-Wallis test; df = degrees of freedom; NS = not significant ($P > 0.05$). The IFA and IHA values are means ($\log_2$ of two-fold dilutions of serum samples). The EIA values are mean optical densities.

Info software, version 6.04 (Pan American Health Organization/World Health Organization). Analysis of variance or the Kruskal-Wallis test were used to compare geometric mean titers of antibodies against *T. cruzi,* its trend during follow-up, and the relationship between antibody titers in each evaluation and baseline estimates. Chi-square or Fisher's exact tests were used to compare differences between the BTCh and PTCh groups in the frequency of side effects, abnormal ECG, and the proportion of those with positive xenodiagnosis. The MacNemar test was used to evaluate seroconversions (from seropositive to seronegative) in the BTCh and PTCh groups.

RESULTS

One hundred six children completed the treatment under the study protocol. The treatment was well tolerated. Less than 20% of participants reported side effects that included intestinal colic, cutaneous maculopapular rush, headache, anorexia, vomiting, nausea, diarrhea, dizziness, paresthesia, and light shivering of the hands. During treatment only intestinal colic and rush were more frequent in the BTCh group than in the PTCh group ($P < 0.05$), appearing at days 11.3 (SD = 9.6) and 19.5 (SD = 16.7), respectively. No severe side effects were observed during treatment. Six (10%) of 55 in the BTCh group had moderate side effects. All side effects disappeared when the treatment was suspended. The BTCh and PTCh groups did not differ significantly in the results of the clinical laboratory examinations. No child showed clinical manifestations during the follow-up. At baseline, ECG examinations showed some abnormalities (left anterior hemiblock or right bundle branch block level II) in 5.0% of the BTCh group and 4.8% of the PTCh group ($P > 0.05$). After 48 months, changes in the ECGs were detected in 2.5% (1 of 40) of the BTCh group and in 2.4% (1 of 41) of the PTCh group ($P > 0.05$); both were ventricular ectopic beats. After 48 months, no disturbances in the electric conduction (blocks) were detected.

Only 101 (95.2%) children that completed more than 30 days of treatment underwent serologic analysis. The BTCh group showed a significant decrease in IHA and IFA geometric mean titers and EIA optical densities, whereas no significant change was observed in the PTCh group (Table 1). Seroconversion to a negative result after 48 months of follow-up was detected with conventional serology in 11.3% (5 of 44) of the BTCh ($P < 0.05$, by the McNemar test) in 4.5% (2 of 44) of the PTCh group ($P > 0.05$, by the McNemar test). The percentage of the BTCh group that tested seronegative by the F29 EIA increased from 35.7% to 62.1% six and 48 months, respectively, after treatment. No placebo-treated child seroconverted to a negative result by the end of follow-up. Figure 1 shows the percentage of children seroreactive for *T. cruzi* during the follow-up. Nearly 38% of the BTCh group and 100% of the PTCh group were seropositive for *T. cruzi* at the end of follow-up ($P < 0.001$). Xenodiagnosis carried out at this time was positive in 51.2% of the PTCh group and in 4.7% of the BTCh group ($P < 0.001$) (Figure 2). All children in the PTCh group were seropositive for *T. cruzi* by the F29 EIA, including those with a positive xenodiagnosis. All children in the BTCh group with a positive xenodiagnosis after follow-up had a seropositive result by the F29 EIA.

DISCUSSION

These results show the efficacy of the specific treatment with benznidazole against *T. cruzi* in children less than 13 years of age in the indeterminate phase of Chagas' disease, who most likely contracted the infection more than six years before beginning treatment.

Assessment of cure in patients chronically infected with *T. cruzi* and treated is controversial, mainly because of the
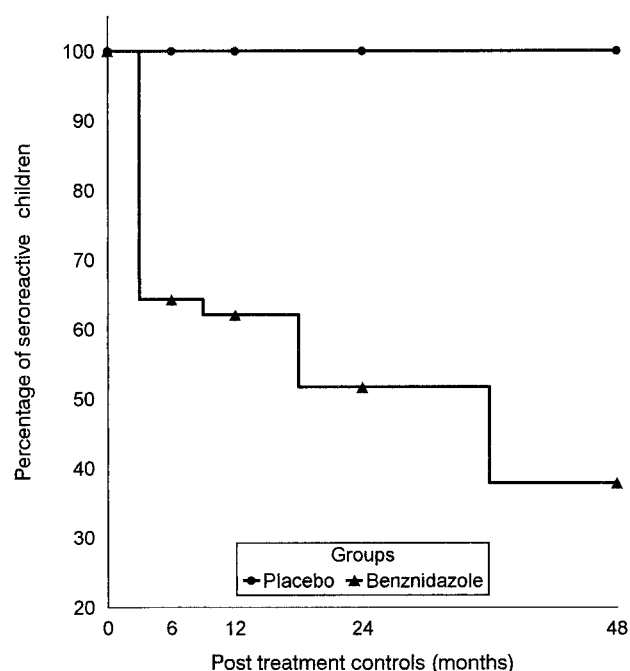
FIGURE 1.   Decrease in the percentage of children with reactive serology against *Trypanosoma cruzi* (indeterminate phase of Chagas' disease) by enzyme immunoassay using the F29 protein after treatment with benznidazole or placebo in Salta, Argentina, 1991–1995.
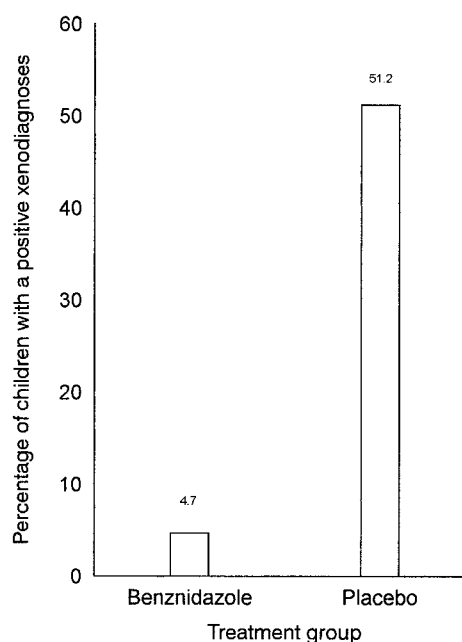

FIGURE 2.   Percentage of children with a positive xenodiagnosis 48 months after treatment with beznidazole or placebo in Salta, Argentina, 1991–1995.

lack of a sensitive and specific test to document parasitologic cure.[10] Most reported trials have included adult patients in the late chronic phase of the disease,[10–12] used different designs and tests to evaluate the tolerance and effectiveness of treatment, and showed different results.

Serious side effects, such as allergic dermopathy, peripheral neuropathy, and granulocytopenia, have been reported in patients treated with benznidazole.[13] The tolerance found in our study using a defined therapeutic regimen of benznidazole in children from six to 12 years of age was good. No severe side effects were found in the BTCh and PTCh groups. Similar results were reported in children up to 12 years of age[14] (Blanco S, Servicio Nacional de Chagas, Córdoba, Argentina, unpublished data).

Other studies have reported cure rates in young adults from 6.0% to 20.0%,[11,12] with changes of antibodies titers assessed by conventional serology. Our results using conventional serology in the BTCh group reported 11.3% seronegative children and a decrease in geometric mean antibody titers after the 48-month follow-up, which is consistent with an independent study carried out in Brazil that followed our protocol, although only for 36 months of follow-up after treatment.[14]

The disappearance of antibodies reacting with the F29 antigen after six months of treatment could be an earlier marker of cure of treated children in addition to conventional serology. Other investigators obtained similar results using other antigens not used in conventional serology.[10,14,15] This is the first study that uses a defined recombinant molecule as antigen[8] in an EIA to evaluate the effectiveness of a treatment against *T. cruzi*.

The persistence of positive results by conventional serol-

ogy for several years after treatment may be due to anti-idiotype reactivity and oscillating nonparasitic stimuli.[16] However, conventional serologic test results may likely revert to seronegative many years after treatment.

The BTCh group had a positive xenodiagnosis less frequently than the PTCh group after a 48-month follow-up. This effect was due to the treatment, as observed in adults patients.[12,17,18]

A hypothesis of a low frequency of positive xenodiagnosis would indicate a low parasitemia and thus a lower antigenic load. This phenomenon would be responsible for preventing the evolution to chronic disease. In support of this hypothesis, clinical studies have demonstrated that chemotherapy with benznidazole in the chronic phase of infection reduced the generation of visceral lesions and decreased antibody titers after eight years of treated.[12] In our study, the follow-up was only for 48 months and the patients were younger; thus, abnormalities in ECGs were uncommon and did not differ between the BTCh and PTCh groups.

Our study demonstrated that children infected with *T. cruzi* that inhabited rural areas may be successfully treated with benznidazole as outpatients. There are good opportunities for administering specific treatment and curing the infection in the first decades of life.[14,19,20] The cure of infection will also reduce the risk of developing visceral alterations[12] and contribute to the interruption of *T. cruzi* transmission in areas under vector surveillance. Moreover, our study provides a new serologic marker of cure after treatment implemented in a rapid and simple serologic procedure.

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