SHORT REPORT: BENZNIDAZOLE EFFICACY AMONG TRYPANOSOMA CRUZI-INFECTED ADOLESCENTS AFTER A SIX-YEAR FOLLOW-UP

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Abstract. Data from a six-year follow-up of Trypanosoma cruzi-infected adolescents enrolled in a randomized, double-blind, clinical trial of benznidazole showed successful chemotherapy in 64.7% (95% confidence interval [CI] = 50.2–78.7) and 84.7% (95% CI = 66.8–92.9), respectively, by intention-to treat and by per protocol analysis measured by seronegativity in a chemiluminescent enzyme-linked immunosorbent assay with a purified trypomastigote mucin antigen. No incident case of cardiomyopathy was detected by electrocardiogram assessment in this cohort of adolescents who had been infected in childhood. The persistent and consistently long-term negative serologic reactions suggest the absence of the parasite in the treated patients and reinforces the recommendation of early benznidazole chemotherapy for T. cruzi-infected infants as a public health policy in endemic areas.

While the elimination of Chagas disease has been considered a reasonable public health goal, controversies remain about the efficiency of trypanocidal chemotherapy, especially in chronic asymptomatic individuals. Currently, there is a need to find appropriate drugs or therapeutic regimens with existing drugs that can be used in Trypanosoma cruzi–infected individuals to prevent development of severe clinical forms of Chagas disease. In a previous randomized, double-blind, clinical trial carried out among infants in Brazil, we showed that a 60 day-course of benznidazole in patients with early chronic infections with T. cruzi was safe and effective in 55.8% of the children, as shown by seronegative conversion of specific antibodies after a 38-month follow-up, when compared with the placebo group. In addition, a short-term effect of benznidazole on the prevention of cardiac damage was also observed. A similar efficacy (62%) for benznidazole was also observed in a study conducted in Argentinean children.

Recommendations derived from both studies included the need to administer benznidazole to the placebo group and also called for further evaluation to monitor the cure assessment in an extended follow-up, as well as to ascertain the benefits of parasitologic treatment to lessen morbidity. The length of time since drug intake (60 days), the 36 months of follow-up, data processing, and analysis and the acquisition of benznidazole tablets by the Brazilian Chagas Disease Control Program for treating the placebo group was 72 months. At that point, adolescents who had been assigned to the placebo group were also treated with benznidazole (7.5 mg/kg twice a day). In addition, blood samples were collected from both the benznidazole and placebo groups for serologic testing and electrocardiographs (ECG) were recorded to obtain six-year post-randomization measurements of outcomes. Herein, we present results of the efficacy of benznidazole using a highly specific and sensitive chemiluminescent enzyme-linked immunosorbent assay with a trypomastigote mucin antigen (A&T CL-ELISA). The first clinical trial was conducted in accordance with human subjects protocols reviewed and approved by the National Ethical Committee (Brazilian National Ethics Commission–Ministry of Health) and by the Regional Ethical Committee (Federal University of Goiás–Ministry of Health). The extended follow-up was also reviewed and approved by the same institutions. Informed consent for the participants was obtained from the legal guardians of minors.

Participants in the present study were the same as described previously. Briefly, in 1991, 129 schoolchildren (7–12 years old) were randomly allocated to receive a benznidazole (60-day course) or placebo preparation. All children had four positive T. cruzi serologic test results (indirect hemagglutination, indirect immunofluorescence, ELISA, and A&T CL-ELISA). After six years, the A&T CL-ELISA and ECG were repeated blindly. Fifty-three (82.8%) of 64 participants allocated in the treatment group were evaluated and 46 (70.8%) of 65 from the placebo arm completed the follow-up (Figure 1). The analysis was done according to both a per protocol (including only compliers) and intention-to-treat approach (including all children who received at least one week of treatment). The efficacy of benznidazole was calculated as 100 × (1 - RR), where RR is the ratio of children with negative seroconversion in the benznidazole group to the corresponding number of children in the placebo group. Logarithmic, power and exponential regression curves of the A&T CL-ELISA data were obtained using Cricket Graph software version 1.0 (Computer Associates International, Inc., Islandia, NY).

As shown in Figure 1, 33 of the 37 children who had received benznidazole chemotherapy and were seronegative after three years remained seronegative after six years, whereas 14 of 21 children shifted from seropositive to seronegative, resulting in 47 cases with negative seroconversion and 6 with seropositive response. In the placebo arm, of 52 individuals who were seropositive after three years of follow-up, 32 remained positive after six years, whereas 11 showed seroconversion. At the end of follow-up, the adolescents ranged in...
age from 14 to 19 years. No statistical difference was found between individuals lost to follow-up allocated to the benznidazole (n = 115) and placebo (n = 19) groups, even after stratifying for sex and age. The cure rate of the treated group measured as negative A&T CL-ELISA seroconversion was 88.7% (47 of 53), whereas 26.1% (12 of 46) seroconversion occurred in the placebo group, including four with borderline results (P < 0.001). The efficacy of benznidazole by per protocol and by intention-to-treat analysis was 84.7% (95% confidence interval [CI] = 66.8–92.9) and 64.7% (95% CI = 50.2–78.7), respectively (Table 1). The median A&T CL-ELISA titer for the benznidazole-treated group was 0.151 (negative result), while it was strongly positive (1.997) for the placebo group after the six-year follow-up. Treatment monitoring could be followed by plotting the A&T CL-ELISA titers of individual sera using either a logarithmic, power, or exponential function chosen according to the best curve fit. Nine representative cases from the two groups were selected (Figure 2). In general, patients with negative seroconversion had high, significant correlations. For example, in six representative cases treated with benznidazole shown in Figure 2 (top panels), the r values ranged from 0.945 to 0.989. Other treated patients had similar distributions of titers. In contrast, the majority of placebo-treated patients typically showed almost invariable A&T CL-ELISA titers throughout the follow-up (Figure 2, bottom panels).

This Brazilian cohort represented a unique opportunity to assess the efficacy of benznidazole with prolonged monitoring of the patients. The results showed that seronegative conversion occurred in nearly 90% of the treated patients while the antibody levels were stable among untreated individuals during the six-year-follow-up. Based on our findings, benznidazole achieved an efficacy of at least 64%, which is higher than that observed in our previous three-year evaluation (55.8%). However, of greater importance is the persistence of negative A&T CL-ELISA titers among benznidazole-treated patients, in addition to the remarkable increase in the number of cases of negative seroconversion after six years (14 of 19 individuals). Conversely, only 11 of 52 individuals in the placebo group showed negative seroconversion for specific antibodies.

The concept that post-treatment negative serology corresponds to parasitologic cure is supported by recent results obtained with this same cohort, which have shown consistency between the decrease of antibody titers and significantly lower T. cruzi parasitemia measured by a polymerase chain reaction in the benznidazole group (39.6%) compared with the placebo group (64.2%) three years after treatment. No incident case of ECG abnormality was found in this extended evaluation, although early development of cardiomyopathy (complete right bundle branch block) was detected in five children (four in the placebo group) after three years of treatment. Since the age of infection in individuals in this cohort was early, our findings indicate a low frequency of disease progression with cardiac involvement after treatment with benznidazole. Nevertheless, a more comprehensive understanding of the progression of heart pathology in Chagas’

### Table 1

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>A&amp;T CL-ELISA</th>
<th>Efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Total followed-up</td>
</tr>
<tr>
<td>Benznidazole</td>
<td>6</td>
<td>47</td>
</tr>
<tr>
<td>Placebo</td>
<td>34</td>
<td>12</td>
</tr>
</tbody>
</table>

*A&T CL-ELISA = chemiluminescent enzyme-linked immunosorbent assay with a purified trypomastigote mucin antigen; CI = confidence interval.
disease can be explored by risk factor analysis relating probable age at infection, parents and siblings serology and ECG findings, and molecular biology determinations.

In conclusion, this study shows evidence of successful chemotherapy with benznidazole among children and adolescents in extended follow-up monitoring, and reinforces the need for early diagnosis and systematic treatment of *T. cruzi*-infected infants.

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