

Randomised trial of efficacy of benznidazole in treatment of early *Trypanosoma cruzi* infection

Ana Lucia S Sgambatti de Andrade, Fabio Zicker, Renato Mauricio de Oliveira, Simonne Almeida e Silva, Alejandro Luquetti, Luiz R Travassos, Igor C Almeida, Soraya S de Andrade, João Guimarães de Andrade, Celina M T Martelli

Summary

Background Benznidazole, a nitroimidazole derivative, has been recommended for the treatment of acute and congenital *Trypanosoma cruzi* infection (Chagas' disease). We have examined the safety and efficacy of this drug in the treatment of the early chronic phase of *T cruzi* infection.

Methods Between 1991 and 1995, we carried out a randomised, double-blind, placebo-controlled trial in a rural area of Brazil with endemic Chagas' disease. 82% of 2434 schoolchildren (aged 7–12 years) identified in a census were screened for antibodies to *T cruzi* by indirect immunofluorescence, indirect haemagglutination, and ELISA. 130 were positive in all tests and were randomly assigned benznidazole (7.5 mg/kg daily for 60 days by mouth) or placebo. The primary endpoint for efficacy was the disappearance of specific antibodies (negative seroconversion) by the end of 3-year follow-up. The secondary endpoint was the reduction of antibody titres on repeated serological tests. One child moved away from the area just after randomisation and was excluded from the analyses. Insecticidal measures were taken throughout the trial to reduce the risk of reinfection.

Findings Minor side-effects requiring no specific medication were recorded in a small proportion of individuals. On a chemiluminescent ELISA with purified trypomastigote glycoconjugate, serum from all participants was positive at the beginning of the trial. At the end of follow-up, 37 (58%) of the 64 benznidazole-treated participants and 3 (5%) of those who received placebo were negative for *T cruzi* antibodies. The efficacy of benznidazole treatment estimated by intention to treat was 55.8% (95% CI 40.8–67.0). At the end of follow-up, children who received benznidazole had five-fold lower geometric mean titres by indirect immunofluorescence than placebo-treated children (196 [147–256] vs 1068 [809–1408], $p < 0.00001$).

Interpretation The trial showed that a 60-day course of benznidazole treatment of early chronic *T cruzi* infection

was safe and 55.8% effective in producing negative seroconversion of specific antibodies. The results are very encouraging and justify the recommendation of treatment for seropositive children as public health policy.

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Introduction

American trypanosomiasis or Chagas' disease is a chronic disease caused by the parasite *Trypanosoma cruzi*. The disease is transmitted by a triatomine insect vector and also by blood transfusion and transplacentally. The infection may cause an acute self-limited disease, which evolves to a symptomless period, known as indeterminate phase. Several years after infection about 30% of individuals present clinical evidence of heart disease, and around 8% develop megavisceras. However, geographical variations in the frequency of the different clinical forms and in severity have been reported.¹ Chagas' heart disease is one of the main causes of disability and death in many Latin American cities. The control activities implemented in the endemic countries are based on elimination of the insect vector from houses, improvements in housing, and promotion of universal blood screening among blood donors. Seroprevalence rates among young children have fallen from 28% to 1% during the past 15 years, but official estimates indicate that more than 15 million people in South America are infected.^{2,3}

Infection is thought to be life-long, though detection of parasitaemia during the chronic phase is very difficult. Specific chemotherapy with benznidazole or nifurtimox has been recommended for treatment of acute and congenital infection, as shown by the clearance of parasitaemia and the disappearance of antibodies to *T cruzi* (negative seroconversion).^{4,5} The effect of treatment in the chronic phase is controversial and difficult to demonstrate because there are no specific criteria for success during this phase.⁶ Clinical trials with drugs including nifurtimox, allopurinol, and benznidazole did not show any effect of treatment in preventing the development of chronic Chagas' disease.^{3,4,7} The general assumption, however, is that the earlier the diagnosis is made and the specific treatment initiated, the greater the chance of parasitological cure.⁷

Before treatment of infected children as a public health measure can be recommended, full-scale investigation of drug safety and efficacy in this target group, preferably under field conditions,⁸ is essential. An effective treatment might prevent the progression of infection to disease and its complications. Large-scale treatment might also decrease the pool of infected individuals in the population, thus reducing the risk of transmission.

We report the results of a phase III randomised double-blind placebo-controlled field trial of benznidazole in the early chronic phase of *T cruzi* infection carried out from 1991 to 1995 in an endemic area in Brazil.

Departments of Community Health

(Prof A L S Sgambatti de Andrade MD, R M de Oliveira MD, S Almeida e Silva MD, S S de Andrade MD, C M T Martelli MD),

Parasitology (A Luquetti MD), and Tropical Medicine

(J Guimarães de Andrade MD), Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Goiás, Brazil; Communicable Disease Program, Pan American Health Organization—WHO, Washington DC, USA (F Zicker MD); and Department of Microbiology, Immunology, and Parasitology, Universidade Federal de São Paulo, São Paulo, Brazil (Prof L R Travassos MD, I C Almeida PhD)

Correspondence to: Prof Ana Lucia S Sgambatti de Andrade, Departamento de Saúde Coletiva, Instituto de Patologia Tropical e Saúde Pública, Rua 1136, N 630, Setor Marista, 74180–150, Goiânia, Goiás, Brazil

Methods

The participants were recruited in three small communities in the north of Goiás—Posse (25 295 inhabitants), Simolândia (6242 inhabitants), and Guarani de Goiás (4766 inhabitants)—in central Brazil. These areas have endemic Chagas' disease with high transmission rates and seroprevalence rates as high as 30% in the past decade.⁹ Details of the socioeconomic characteristics of the region have been reported elsewhere.^{10,11} Demographic characteristics of the schoolchildren attending 60 village schools in the study area were obtained in the Secretary of Education and Chagas' Disease Control Program.

82% of the 2434 schoolchildren identified in a census were included in a serosurvey carried out from March to September, 1991. The study protocol was ethically and technically reviewed and approved by the Regional Medical Council in accordance with WHO guidelines for biomedical research. Signed informed consent was obtained from the parents or guardians of each child. If the trial showed that benznidazole treatment was successful, the research team would offer free treatment to the children assigned to the placebo group.

Blood samples collected by fingerprick on filter paper were eluted and processed by serological tests (indirect immunofluorescence, indirect haemagglutination, and ELISA)¹² in parallel (figure 1). Details of this screening and the laboratory techniques and cut-off points adopted have been described elsewhere.¹³ To be eligible for the trial, children had to be seropositive by all three tests. 1681 were negative on all three tests and 158 were positive on only two tests (none was positive on a single test). Of the 151 eligible children, 135 were available for confirmatory tests on serum samples collected by venepuncture. 130 children were seropositive by the three tests on these samples. The criteria for a child to be classified as seropositive and included in the trial were a reciprocal titre on indirect immunofluorescence of 40 or more, an ELISA index of 1.2 or more, and a reciprocal titre on indirect haemagglutination of 16 or more. We also used a highly sensitive and specific chemoluminescent ELISA.¹⁴ This test is based on the reactivity of serum with a purified mucin-like glycoconjugate anchored by glycosyl-phosphatidylinositol from cell-cultured trypomastigotes of the Y strain¹⁵ (antigen trypomastigote [AT] ELISA). The method described previously¹⁶ was modified to use direct luminometer readings of the ELISA plates. Serum samples were diluted 1 in 2000 and the titres were expressed as the ratio of the luminometer reading to the cut-off value (defined as ten times the negative control mean minus the background control mean for each plate). Positive serum samples gave ratios greater than 1.0. The various serological tests were done without knowledge of the codes and other results by different investigators at the WHO Reference Laboratory for Chagas' disease serology, Federal University of Goiás.¹⁷ The AT ELISA was carried out at the Federal University of São Paulo.

Clinical examination and laboratory tests were done for all 130 potential trial participants. 12-lead electrocardiograms (ECG) were recorded at rest. Blood samples were collected for leucocyte counting, and measurement of packed-cell volume, serum aspartate and alanine aminotransferases, urea nitrogen, and creatinine. No child was excluded from the trial because of abnormal results.

We enrolled 130 symptom-free children with antibodies to *T cruzi* in all four serological tests carried out on venous blood samples. The randomisation was done in blocks of six children within each school, after stratification for sex and age. One child (assigned benznidazole) moved away just after the randomisation process. 100 mg tablets of benznidazole (Roche, Brazil) were reformulated into 50 mg tablets by the Goiás State Chemical Industry for prescription to children. Placebo was formulated with the same composition as the drug carrier except for the active compound and packaged in matching tablets and vials. The contents of both products were independently analysed for chemical quality and control and approved by the Ministry of Health National Reference Laboratory. Benznidazole and placebo were given at a dose of 7.5 mg per kg bodyweight (fixed dose per

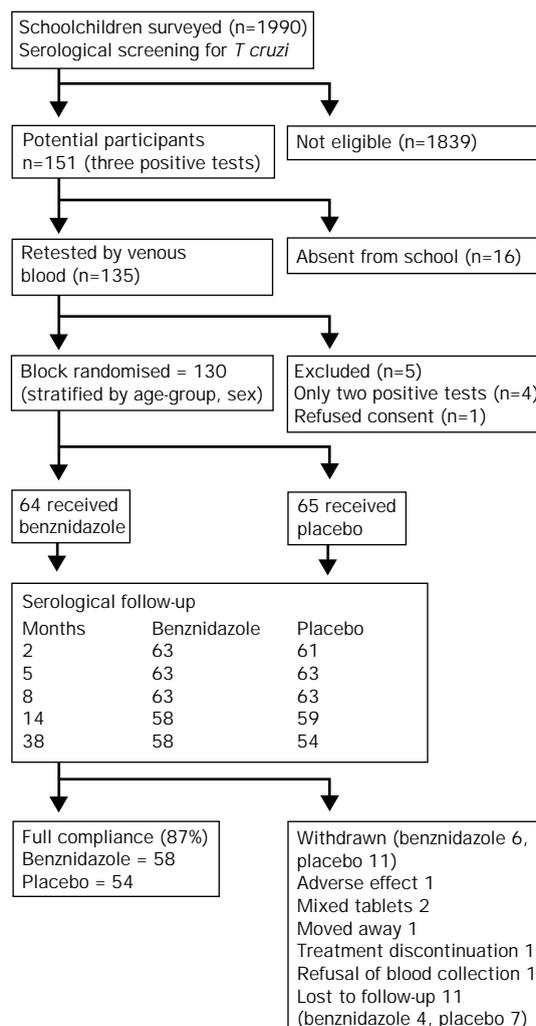


Figure 1: Trial profile

weight classes of 5 kg) divided in two daily doses for 60 days.

Tablets sufficient for the 60-day treatment were put in individual small plastic bags by an independent collaborator, labelled with the names of the child and his or her school and the daily dose to be administered. The drugs, stored at the school, were administered by the classroom teacher at the beginning and end of the school day. During the weekends the children received the exact number of tablets corresponding to the Saturday and Sunday doses for self-administration. Treatment compliance during the weekend was assessed by counting of the remaining pills on the next school day. Children absent from school were visited at home to be treated. Permanent medical supervision was maintained during the treatment period to ensure compliance and to monitor side-effects and toxicity. Sealed security envelopes containing each individual assignment to benznidazole or placebo were held by the formulation department of the Goiás State Chemical Industry.

During follow-up, toxic effects were assessed through haematological examination (white-cell count and haemoglobin), liver and renal function (aspartate and alanine aminotransferases, urea nitrogen, and creatinine), and clinical examination before treatment and at days 7, 14, and 45 of treatment. A physician visited the schools daily for a brief interview with each child. Side-effects were recorded with special attention to clinical manifestations known to be associated with benznidazole use, such as gastrointestinal symptoms, headache, rash, itching, arthralgia, and peripheral-nerve involvement (hyperaesthesia and pain sensation).^{4,18}

Serum samples were taken on the last day of treatment (day 60) and 3, 6, 12, and 36 months after completion of treatment.

	Benznidazole (n=64)	Placebo (n=65)
Male/female	38/26	38/27
Age group		
7-9 years	24	22
10-12 years	40	43
ECG abnormalities*	6	7
Serological results (95% CI)		
Indirect immunofluorescence†	1605 (1351-1911)	1634 (1370-1950)
Indirect haemagglutination†	202 (147-274)	267 (194-362)
ELISA‡	2.7 (2.6-2.9)	2.9 (2.7-3.0)
Mean (SD; range) haematological measures		
Packed-cell volume (%)	36.6 (2.7; 30-42)	37.1 (2.7; 32-48)
Leucocyte count (x10 ⁹ /L)	8.71 (4.97; 3.80-39.4)	7.86 (2.98; 3.20-17.0)
Neutrophil count (x10 ⁹ /L)	3.48 (1.95; 0.43-12.2)	3.00 (1.77; 0.96-10.8)
Mean (SD; range) liver function test results		
Serum aspartate aminotransferase (U/L)	19.1 (7.9; 8-38)	19.9 (6.1; 6-38)
Serum alanine aminotransferase (U/L)	15.9 (7.0; 4-37)	16.7 (6.4; 4-30)

*Benznidazole group: 2 complete right bundle branch block; 1 left anterior hemiblock; 1 second-degree atrioventricular block; 1 ectopic rhythm; 1 first-degree atrioventricular block. Placebo group: 7 complete right bundle branch block.

†Geometric mean titre. ‡Mean absorbance.

Table: Baseline characteristics of trial participants

All samples were tested simultaneously by the four serological tests at the end of the trial to avoid observer and process variation.

To avoid the risk of reinfection during follow-up, all dwellings in the study communities were sprayed with deltamethrin by the Chagas' Disease Control Program, before the trial and then every 6 months. About 10% (n=175) of the seronegative children in the community were tested every 6 months for seroconversion. No incident case was detected during the trial follow-up.

The primary endpoint established for the trial was the absence of specific antibodies (negative seroconversion) at the end of a 3-year follow-up period. The secondary endpoint was a three-fold or greater reduction in antibody reciprocal titres assessed through repeated serological tests. No parasitological methods were used to assess treatment efficacy because these techniques have low sensitivity (30-50%) in the chronic phase of the infection.¹⁹

The sample size was estimated to detect a 15-20% negative seroconversion rate in the benznidazole group, at 5% significance with 90% power. The results of the AT ELISA were reported as positive or negative and treated as proportions. To analyse trends in titres of the conventional serological tests, reverse cumulative

frequency distribution curves²⁰ were plotted for each test and treatment group at baseline and at the end of follow-up. ELISA mean absorbance and log mean titres by indirect immunofluorescence and indirect haemagglutination (with 95% CI), were compared between the treatment groups at different times. Geometric mean titres (and 95% CI) were estimated as antilogarithms of the log-transformed mean dilutions. χ^2 tests or Fisher's exact tests were used, as appropriate, to examine differences in the categorical characteristics; *t* tests were used to compare log-transformed antibody titres. For the primary endpoint, the final analysis was done according to the intention-to-treat principle, including all children who received at least 1 week's treatment. Benznidazole efficacy was calculated as $100 \times (1 - RR)$, where RR is the ratio of the proportion of children with negative seroconversion in the benznidazole group to the corresponding proportion in the placebo group (by the AT ELISA).²¹

Results

Figure 1 summarises the trial design and screening procedures for recruitment of participants. The two groups had similar age and sex distributions (table). Although all children were clinically free of symptoms, the ECG recorded at the beginning of the study showed abnormalities in 13. Nine children had complete right bundle branch block which indicates premature development of Chagas' disease cardiomyopathy. The overall frequency of ECG abnormalities did not differ significantly between the groups, and was not judged to contraindicate benznidazole treatment. Serological, haematological, and biochemical results were similar in the two groups.

Serological results were available for the 112 (87%) participants who completed the full treatment and follow-up period. 17 children withdrew from the study (figure 1). The children who withdrew did not differ significantly in age, sex, or antibody titres at baseline between the treatment groups.

Side-effects and toxicity

Benznidazole was well-tolerated; slight adverse effects such as nausea, anorexia, headache, stomach-ache, and arthralgia were reported by less than 5% of participants, with no significant differences between the groups. Cutaneous maculopapular rash and pruritus during the treatment were more common in the benznidazole group

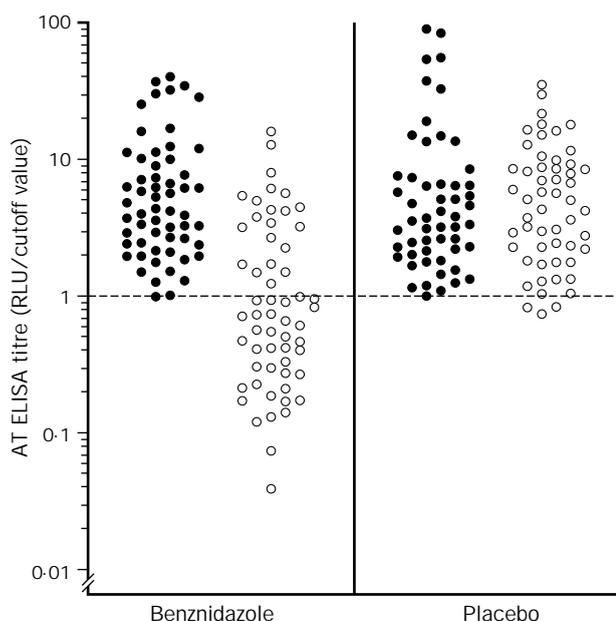


Figure 2: AT ELISA results at trial entry (●) and at end of follow-up (○) for 58 benznidazole-treated and 54 placebo-treated children who completed trial treatment

Broken horizontal line=cut-off; values below this indicate seronegativity.

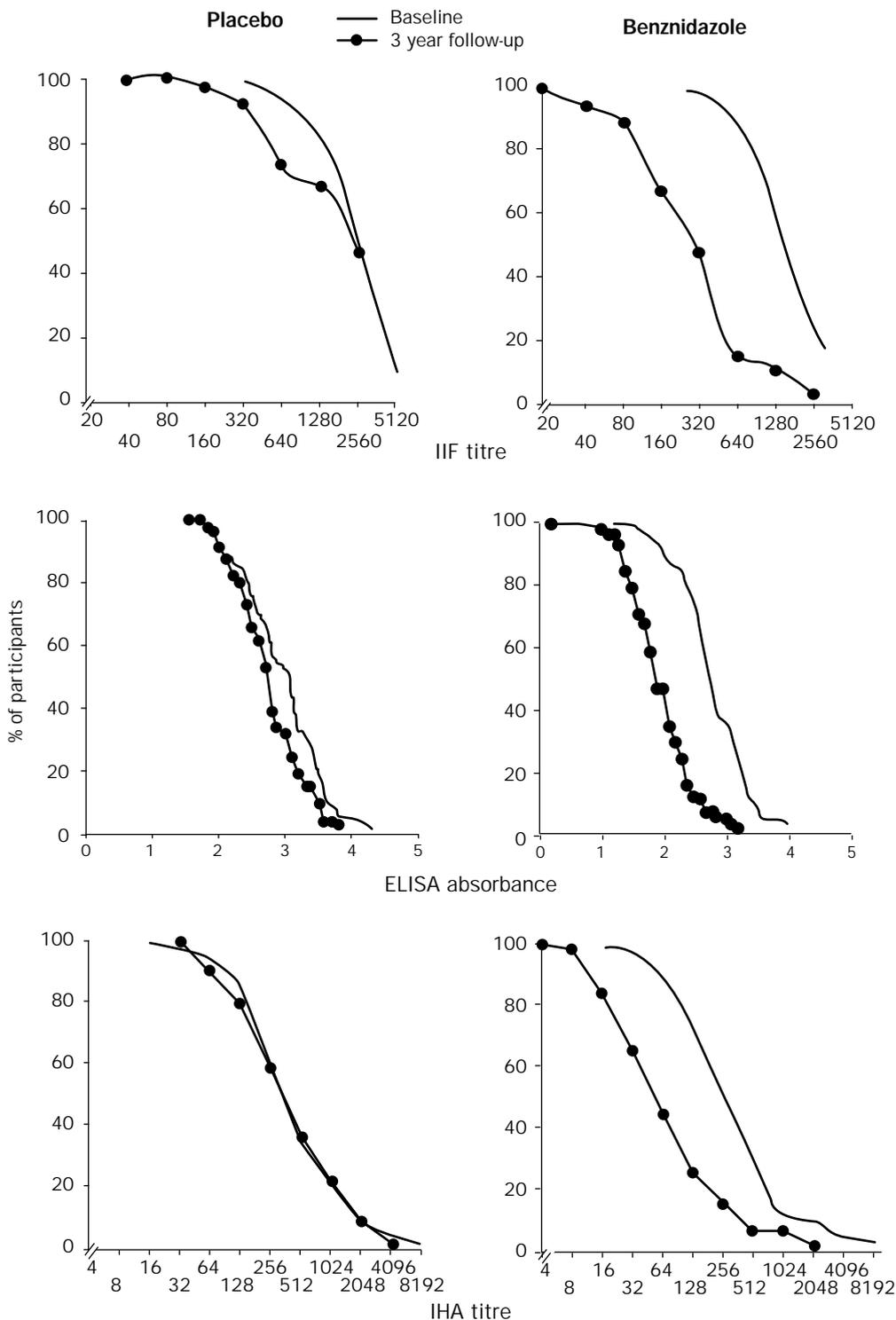


Figure 3: Reverse cumulative distribution curves of titres of antibodies against *T cruzi* among children receiving placebo and benznidazole at baseline and at 3 years of follow-up.

n=58 for benznidazole, 54 for placebo group. IIF= indirect immunofluorescence; IHA=indirect haemagglutination.

than in the placebo group (eight and two cases, respectively; $p=0.05$), this side-effect was the reason for withdrawal of one patient from the benznidazole group.

No sign of toxicity was detected. The frequency of anaemia (haemoglobin ≤ 110 g/L) was similar in the two groups (15.4%) and no patient developed leucopenia (white-cell count $< 0.3 \times 10^9/L$) or neutropenia (neutrophil count $< 0.75 \times 10^9/L$). Results of liver and kidney function tests were within normal limits at baseline and did not

change significantly during the study. ECG recorded at the end of the follow-up showed one (1.7%) incident case of complete right bundle branch block in the benznidazole group and four (6.9%) in the placebo group ($p=0.36$).

Treatment efficacy

Negative seroconversion (disappearance of antibodies to *T cruzi* by the AT ELISA) occurred in 37 of the 58 benznidazole-treated children who completed treatment,

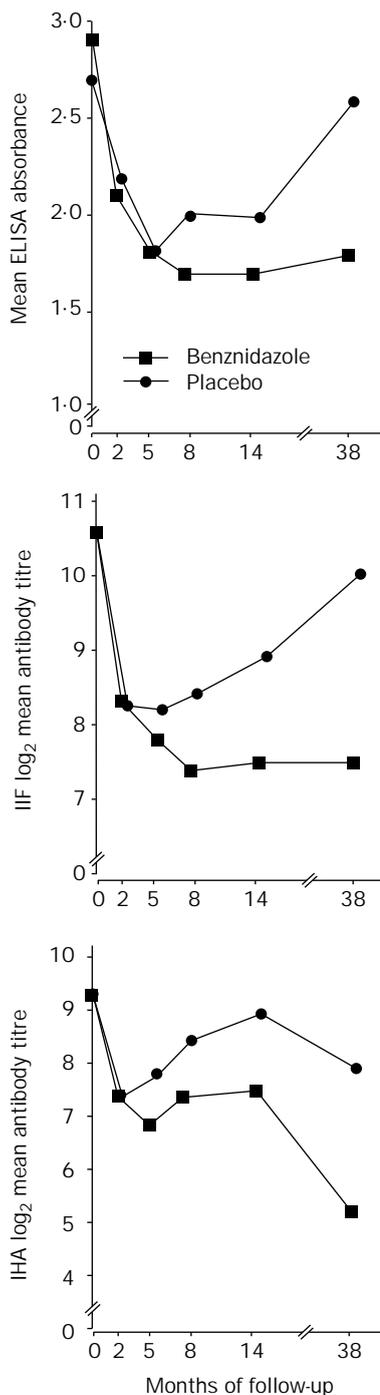


Figure 4: *T. cruzi* serological response in benznidazole and placebo groups by time. Error bars indicate 95% CI. IIF=indirect immunofluorescence; IHA=indirect haemagglutination.

compared with three of the 54 placebo-treated children who completed treatment (figure 2).

Interpretation of the results of conventional serological tests was not so straightforward. The cumulative distribution curves for titres by indirect immunofluorescence and indirect haemagglutination and for ELISA results were constant for the placebo group with overlapping curves over time, whereas the distributions of titres in the benznidazole group shifted to the left, indicating a decline in the proportion of participants with higher titres. For example, at the end of

the trial, by indirect immunofluorescence the median reciprocal titre for the benznidazole group was 320 and that for the placebo group 2560 (figure 3). The analysis of titres over time indicated a consistent decrease in antibody concentrations in the benznidazole group and an initial decrease followed by a progressive increase in the placebo group (figure 4). A significant difference between the groups was apparent 6 months after completion of treatment, when the 95% CI of the mean estimates of the two groups did not overlap. At the end of follow-up, children who received benznidazole had five-fold lower geometric mean titres by indirect immunofluorescence than placebo-treated children (196 [147–256] vs 1068 [809–1408] $p < 0.00001$).

The intention-to-treat analysis based on results of the AT ELISA showed that treatment was successful in 37 (58%) of 64 children in the benznidazole group compared with 3 (5%) of 65 in the placebo group. Children who showed negative seroconversion did not differ from those with persisting antibody in sex or age distribution, but they had significantly lower antibody titres at baseline. The treatment efficacy was estimated as 55.8% (40.8–67.0). No significant changes in the results were observed after adjustment for sex, age, and ECG abnormalities.

Discussion

Specific treatment of Chagas' disease has been recommended only for the acute phase of the infection. Clearance of parasitaemia and disappearance of antibodies are taken as cure criteria.^{4,22} Most reported trials have recruited adult patients under medical care in a late phase of chronic infection.^{23,24} Cure assessment in chronic infection is controversial, mainly because of the lack of sensitive or specific tests to document parasitological cure.^{22,23} Experience with benznidazole started more than 15 years ago.^{4,24,25} One of the few studies of a large number of patients in the indeterminate phase ($n=50$) reported a 10% cure rate compared with a 6% cure rate in a historical comparison group treated with nifurtimox.²⁴ However, the therapeutic results have differed between countries, probably because of differences in *T. cruzi* strains.⁷

Our randomised double-blind placebo-controlled efficacy trial was conducted in children infected with *T. cruzi*, presumably in their first years of infection. This trial cannot be compared with previous studies because of differences in study populations, dosage, outcome measures, and time of follow-up, and because most of those studies lacked a placebo group. Serious adverse reactions have been reported in adults treated with benznidazole, including generalised allergic dermatopathy, peripheral neuropathy, and granulocytopenia.¹⁸ In our trial only one child was withdrawn due to a moderate papular rash during treatment phase. No patient had peripheral-nerve symptoms and there were no haematological or hepatic abnormalities attributable to the drug. These findings are compatible with previous observations of lower toxicity in children than in adults, even with higher doses.⁴

A 60-day course of benznidazole treatment was 55.8% effective in leading to disappearance of specific antibodies, which was used as a surrogate measure of parasite clearance. Although much emphasis has been given to the development of polymerase chain reaction assays for *T. cruzi* treatment evaluation,²⁶ this approach is not yet well established.

The disappearance of lytic antibodies has been advocated as a suitable indicator of parasitological cure.^{6,23} The specificity of the lytic antibodies is related to reacting epitopes presenting glycoconjugate antigenic fractions of the parasite (F2/3), indicators of active infection. Serum from individuals negative for lytic antibodies and positive by conventional serology were also negative in a chemoluminescent ELISA with the F2/3 glycoconjugate,^{16,27} which supports our use of the AT ELISA. This assay proved to be highly sensitive and specific for detecting antibody to *T. cruzi*⁴ and thus for monitoring trypanosomicidal treatment.

The decline in antibody concentrations after chemotherapy is a slow process.^{22,28} The persistence of positive results on conventional serology for some time after treatment may be due to anti-idiotypic reactivity and oscillating non-parasitic stimuli.²⁹ We found striking differences in the antibody concentrations between the benznidazole and placebo groups as early as 6 months after completion of treatment. The 21 subjects in the benznidazole group who did not show negative seroconversion did have significant reductions in antibody concentrations compared with their baseline values. We should emphasise that the children's environment was maintained free of transmission during follow-up.

The randomisation and masking procedures helped to reduce to a minimum selection and ascertainment bias.⁸ Although migration is expected in rural settings, 87% of participants completed treatment and follow-up. In addition, those who withdrew did not differ significantly in sex or age between the treatment groups. Thus, the benznidazole efficacy estimate is unlikely to have been biased by the loss to follow-up.

Although morbidity evaluation was outside the scope of this trial, the detection of five new cases of cardiomyopathy (complete right bundle branch block), four of whom received placebo, indicates early development of cardiac lesions in this study population. Assessment of the preventive effect of benznidazole treatment on progression from infection to disease would be possible only through extended follow-up. In mice, benznidazole treatment was associated with late regression of histopathological lesions of myocardial and skeletal muscle.³⁰

We have previously suggested the regular serological testing of schoolchildren as a surveillance method to identify communities in which transmission is occurring.¹³ This approach has been adopted by the Brazilian Chagas' Disease Control Program and a nationwide serological survey for *T. cruzi* infection has just been completed. At this stage, the benznidazole efficacy shown by this trial and the confirmed safety of the drug justify the recommendation of treatment of seropositive children as public health policy.

Substantial progress has been made in decreasing the vectorial transmission of *T. cruzi* and in elimination of one of the main vectors, *Triatoma infestans*, but little progress has been made in treatment of infected people. The development of new drugs and the evaluation of the impact of the trypanosomicidal treatment in preventing morbidity remain major challenges for Chagas' disease control and health care.

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Risk of aspirin-associated major upper-gastrointestinal bleeding with enteric-coated or buffered product

Judith P Kelly, David W Kaufman, Jan M Jurgelon, Jane Sheehan, Raymond S Koff, Samuel Shapiro

Summary

Background Aspirin products are known to cause irritation and injury to the gastric mucosa. The belief that enteric-coated and buffered varieties are less likely to occasion major upper-gastrointestinal bleeding (UGIB) than plain aspirin was tested in data from a multicentre case-control study.

Methods 550 incident cases of UGIB admitted to hospital with melaena or haematemesis and confirmed by endoscopy, and 1202 controls identified from population census lists, were interviewed about use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) during the 7 days before the onset of bleeding (cases) or interview (controls). Relative risks of UGIB for each type of aspirin used regularly (at least every other day) were calculated overall, and according to dose, by multiple logistic regression, with control for age, sex, marital status, date, education, cigarette smoking, alcohol use, and use of NSAIDs.

Findings The relative risks of UGIB for plain, enteric-coated, and buffered aspirin at average daily doses of 325 mg or less were 2.6, 2.7, and 3.1, respectively. At doses greater than 325 mg, the relative risk was 5.8 for plain and 7.0 for buffered aspirin; there were insufficient data to evaluate enteric-coated aspirin at this dose level. There were no important differences in risk attributable to the three aspirin forms according to bleeding site (gastric vs duodenal), or when users of NSAIDs were excluded.

Interpretation Use of low doses of enteric-coated or buffered aspirin carries a three-fold increase in the risk of

major UGIB. The assumption that these formulations are less harmful than plain aspirin may be mistaken.

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Introduction

Although there have been many reports of upper-gastrointestinal bleeding (UGIB) associated with non-steroidal anti-inflammatory drugs (NSAIDs),^{1–11} these agents continue to be frequently prescribed, and access to their use has increased as some (ibuprofen, naproxen, ketoprofen) have become available over the counter. Grossman¹² has suggested that use of NSAIDs be replaced by use of enteric-coated aspirin, a less expensive and purportedly safer alternative.

Several hypothetical mechanisms by which aspirin could bring about UGIB have been proposed.^{13,14} Local irritation could allow back-diffusion of acid into the gastric mucosa, causing injury to mucosal cells and the submucosal capillaries, followed by necrosis and bleeding. Inhibition by aspirin of cyclo-oxygenase may occur; this enzyme catalyses the synthesis of gastric prostaglandins PGI₂ and PGE₂ which inhibit secretion of acid by the stomach and promote the secretion of cytoprotective mucus. Inhibition of synthesis may therefore render the mucosa more susceptible to damage. There could also be an increased tendency to bleed because of impairment of platelet aggregation by aspirin.

An aspirin tablet coated with a combination of cellulose, silicon, or other inactive ingredients has resistance to disintegration in the stomach; this property allows dissolution of the drug in the more neutral to alkaline environment of the duodenum.¹⁵ The safety of this type of product has been confirmed by several endoscopic studies that compared enteric-coated with plain aspirin preparations in healthy volunteers.^{16–18} There was less gastric erosion and microbleeding in those who used the enteric-coated preparation. Buffering agents (calcium carbonate, magnesium oxide, and magnesium carbonate)

Slone Epidemiology Unit, School of Public Health, Boston University School of Medicine, Brookline, Massachusetts, MA 02146 USA (J P Kelly MS, D W Kaufman ScD, J M Jurgelon MPH, J Sheehan MS, S Shapiro, FRCPE), and Department of Medicine, MetroWest Medical Center, Framingham Union Campus, Framingham, Massachusetts (R S Koff MD)

Correspondence to: Ms Judith P Kelly