TREATMENT OF CHRONIC CHAGAS’ DISEASE WITH ITRACONAZOLE AND ALLOPURINOL

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Abstract. Four hundred four patients with chronic Chagas’ disease were treated with itraconazole (6 mg/kg of body weight/day for 120 days), allopurinol (8.5 mg/kg of body weight/day for 60 days), or with a placebo of pure starch. Patients were monitored over a period of four years by clinical examination, serology, xenodiagnosis, hemoculture, and electrocardiogram. Drug tolerance was good, with only four treatments discontinued due to side effects that subsided after suspension of treatment. Parasitologic cure was evident in 44% of the those treated with allopurinol and 53% of those treated with itraconazole, and the electrocardiographic evaluation showed normalization in 36.5% and 48.2%, respectively, of patients with chronic or recent cardiopathy.

According to the World Health Organization, there are 16–18 million individuals infected with Trypanosoma cruzi (causal agent of Chagas’ disease) in Latin America. More than half of these are in the Southern Cone countries (Argentina, Brazil, Bolivia, Chile, Paraguay, and Uruguay) where a major program is currently underway to halt transmission through large-scale vector control and screening of blood donors. Previous investigations carried out by us in Chile indicated that there are approximately 350,000 infected individuals, 30% of whom have some degree of cardiopathy. Of these, we estimate that about one-third will require a cardiac pacemaker to survive.

In spite of recent advances, there is no drug that provides satisfactory treatment of chronic T. cruzi infections. There are many drugs with activity against T. cruzi, but the only ones that can currently be given to humans on the basis of ethical and clinical considerations are nifurtimox (Lampit; Bayer Chemicals, Leverkusen, Germany), benznidazole (Ragonil; Roche, Basel, Switzerland), and primaqune (Neoquienil; Sanofi Winthrop, New York, NY). Nifurtimox and benznidazole are used successfully in the treatment of acute infections, and complete clinical, parasitologic, and serologic cures are occasionally achieved. Primaquine has been used in acute cases only when there is no access to the other drugs because it induces clinical improvement but not parasitologic cure. In chronic cases, the same drugs have been used, as well as allopurinol, in various dosages and for different lengths of time with variable results. Nifurtimox and benznidazole are now available on a limited basis; however, both have side effects, especially in adults, which limits their use. For these reasons it is important to have access to an efficient, safe, low-cost drug. Itraconazole, a synthetic imidazole derivative, has shown good efficiency in in vitro and in vivo experiments, resulting in total cure in chronically infected mice. This drug has been used in humans for the last 20 years as an efficient antifungal without any severe side effects, and thus merits evaluation for the treatment of chronic Chagas’ disease.

MATERIALS AND METHODS

We chose to evaluate itraconazole by comparison with allopurinol, with which we have previous experience in treating chronic Chagas’ disease infections. The treatment schedule and follow-up protocol were approved by the Ethics Committee of the Medical Research Secretariat, Faculty of Medicine, University of Chile. Patients were informed in detail about the protocol and consented to take part in this study.

A total of 404 individuals with chronic Chagas’ disease (age range = 9–50 years) were randomly assigned to be treated with either itraconazole (135 patients), allopurinol (104 patients), or a pure starch placebo (165 patients). Of this total, 96 patients (23.8%) were from the hypoendemic metropolitan region of Santiago (33°55′S, 69°46′W), 168 patients (41.6%) were from the hyperendemic IV region (29°02′S, 69°49′W), and 140 (34.6%) were from the V region (32°02′S, 70°00′W), which is also hyperendemic for T. cruzi transmission.

Itraconazole in 100-mg capsules was provided by Jansen Laboratories (Beerse, Belgium). Allopurinol in 300-mg tablets was supplied by Silesia Laboratories (Santiago, Chile) as Urogotan® and by Saval Laboratories (Santiago, Chile) as Ziloric®. Saval Laboratories also provided the placebo as 30-mg pure starch tablets.

In the hyperendemic areas, treatment was carried out at the rural outpatient clinics of the municipalities, while in metropolitan Santiago this was done in the Outpatient Clinic of the Department of Parasitology of the Faculty of Medicine, University of Chile, Southern Campus. To make sure that the patients received the drugs, a paramedic supervised that the patients swallowed the tablets. Treatment was double-blind in that neither the investigators who administered the treatment nor the staff involved in data analysis were aware of the original therapy codes.

Prior to treatment, each patient was given a clinical, serologic, and electrocardiographic examination. Serologic diagnosis was made by indirect hemagglutination (IHA), indirect immunofluorescence (IF), and ELISA. In addition, xenodiagnosis was applied using 2–4 boxes of 5–8 third instar nymphs of Triatoma infestans. A Western blot with total antigen (EIT-T) and complement-mediated lysis (CoML) were also performed in 195 patients, and 34 of them were also evaluated by Western blot with excreted-secreted antigens (EIT-S). A 12-derivation electrocardiographic study on all patients followed the double-blind protocol recommended by the World Health Organization. Under this protocol, the investigator analyzing the electrocar-
diagram (ECG) traces remains unaware of the patient’s serology or xenodiagnostic results (or subsequent treatment status). From the initial results, we could include in the study patients with the indeterminate (asymptomatic) form of chronic chagasic infection (i.e., with normal ECG traces) and patients with symptomatic or asymptomatic chagasic cardiopathy (i.e., showing ECG alterations) in whom all other known etiologies had been ruled out (hypertension, atherosclerosis, valvular disease, congenital malformations, myocardopathies, etc.).

Under the randomized treatment schedule (Figure 1), the drugs were allocated to the patients as they came to the clinic (itraconazole, allopurinol, and placebo), except when the supply was exhausted; placebo was administered to 165 of the 404 individuals: 129 (78.2%) of the indeterminate group and 36 (21.8%) of those with chagasic cardiopathy. A daily allopurinol dose of 8.5 mg/kg of body weight was administered by the oral route for 60 days. After completion of administration of the placebo, these patients were then randomly assigned to receive either itraconazole (82 patients) or allopurinol (83 patients). However, of the 82 patients reassigned to receive itraconazole, only 20 (24.4%) completed the subsequent treatment. This was partly due to the reluctance of some patients to continue a further supervised treatment of 120 days required for itraconazole, and partly because some of these patients relocated their area of residence and became unavailable for continued treatment.

Itraconazole was thus administered to a total of 155 patients (20 of whom had received placebo during the preceding two months). Of these, 110 (70.9%) were in the indeterminate group and 45 (29%) had chagasic cardiopathy. The oral dose was 6 mg/kg of body weight/day, divided into two doses, for 120 days. Allopurinol was administered to 185 individuals (81 of whom had received placebo during the preceding two months). Of these, 127 (69%) were in the indeterminate group and 58 (31%) had chagasic cardiopathy. Allopurinol was given orally at a dosage of 8.5 mg/kg of body weight once a day for 60 days. Thus, the final treatment groups were as follows: 165 placebo, 155 itraconazole, and 185 allopurinol, representing 505 treatments (Figure 1).

To monitor for possible side effects of treatment, the following laboratory tests were performed before treatment and then once every month until 30 days after completion of therapy: bilirubin, total cholesterol, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase. Drug tolerance was considered satisfactory when there were no secondary signs or symptoms imputable to the drugs and laboratory test results remained normal. Tolerance was considered moderate when mild signs or symptoms were present and/or minimal alterations of the laboratory test results appeared. Tolerance was considered unsatisfactory when severe secondary signs or symptoms were observed and/or important alterations of laboratory tests results became evident, which mandated interruption of therapy.

The outcome of treatment was evaluated every four months with IHA, IF, ELISA, CoML, EIT-T, and EIT-S tests, together with clinical and electrocardiographic examinations, with four years of follow-up. Xenodiagnosis was performed immediately after treatment and every six months for the first two years, and once per year during the last two years. Hemocultures were obtained from those patients whose xenodiagnosis remained negative after treatment. The method of Chiari and others was used for the hemocultures: 20 ml of blood was collected into tubes containing heparin and centrifuged for 10 min at 300 g at room temperature. The packed red blood cells were washed twice by centrifugation in liver infusion tryptose (LIT) culture medium at 900 g for 30 min, and divided among six tubes, each containing 3 ml of LIT. All tubes were maintained at 28°C and examined microscopically every month for up to 120 days.

Criteria for parasitologic cure after treatment were negative results for xenodiagnosis and/or CoML maintained throughout the four-year follow up. Electrocardiographic improvement was defined by normalization of a previously altered ECG tracing persisting for at least four years. Results were statistically analyzed using multiple proportions and Fisher’s exact and chi-square tests.

RESULTS

Epidemiologic background. The epidemiologic survey demonstrated that the majority of patients from the hypoen-
ELISA, and Western blot results remained positive without variations in these cases. Patients who received placebo did not show changes in serologic titers.

Results of xenodiagnosis in the 501 patients treated with itraconazole, allopurinol, or placebo who completed their follow-up are shown in Table 2. Of 43 treatments with itraconazole in patients who had positive xenodiagnosis before therapy, the test result became negative in 38 (88.4%). Of the 29 patients with positive xenodiagnosis before treatment with allopurinol, the test result became negative in 18 (62%); on the other hand, in the group who received placebo, the xenodiagnosis result became negative in only four (25%) of 16. In terms of the xenodiagnosis results, comparisons between groups treated with itraconazole, allopurinol, and placebo were statistically significant (P < 0.05).

In the 111 treatments with itraconazole who had negative xenodiagnosis before therapy, the test result remained negative in 109 (98.2%). Of the 153 cases with negative xenodiagnosis who were treated with allopurinol, the test result remained negative in 149 (97.4%). Of the 149 treatments with placebo whose xenodiagnosis was negative before therapy, the results did not change in 142 (95.3%).

Evolution of electrocardiography. Table 3 shows the ECG changes in 103 chagasic disease patients with cardiopathy treated with either itraconazole or allopurinol. The ECG returned to normal in 39 cases (36.5%). Twenty-five cases with prolonged QTc syndrome also showed a return to normal results. Among the alterations of intraventricular conduction, 10 unifascicular blocks disappeared, mainly left anterior hemiblocks and two bifascicular blocks (left anterior hemiblock and right bundle branch block). One tracing suggestive of ischemia and three showing left ventricular hypertrophy also improved. The difference between the initial and final prolonged QTc was statistically significant (P < 0.01, by Fisher’s exact test). In relation to atrioventricular blocks, a first degree A-V block and a third degree A-V block appeared, with the latter requiring a pacemaker implant; these patients had received allopurinol and itraconazole, respectively.

### Table 1
Tolerance to itraconazole, allopurinol, and placebo in 505 patients with chronic Chagas’ disease

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Tolerance</th>
<th>Itraconazole</th>
<th>Allopurinol</th>
<th>Placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Satisfactory</td>
<td>139</td>
<td>89.7</td>
<td>167</td>
<td>90.3</td>
<td>154</td>
</tr>
<tr>
<td>Moderate</td>
<td>15</td>
<td>9.7</td>
<td>15</td>
<td>8.1</td>
<td>11</td>
</tr>
<tr>
<td>Unsatisfactory</td>
<td>1</td>
<td>0.6</td>
<td>3</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>155</td>
<td>100.0</td>
<td>185</td>
<td>100.0</td>
<td>165</td>
</tr>
</tbody>
</table>

### Table 2
Results of xenodiagnosis in 501 patients treated with itraconazole, allopurinol, or placebo

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Xenodiagnosis</th>
<th>Itraconazole</th>
<th>Allopurinol</th>
<th>Placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Total</td>
<td>%</td>
<td>No. Total</td>
<td>%</td>
<td>No. Total</td>
</tr>
<tr>
<td>+ −</td>
<td>38</td>
<td>43</td>
<td>88.4*</td>
<td>18</td>
<td>29</td>
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<td>+ +</td>
<td>5</td>
<td>43</td>
<td>11.6</td>
<td>11</td>
<td>29</td>
</tr>
<tr>
<td>− −</td>
<td>109</td>
<td>111</td>
<td>98.2</td>
<td>149</td>
<td>153</td>
</tr>
<tr>
<td>− +</td>
<td>2</td>
<td>111</td>
<td>1.8</td>
<td>4</td>
<td>153</td>
</tr>
</tbody>
</table>

*P < 0.05, by difference of proportions test. Itraconazole > allopurinol > placebo.
was statistically significant (The difference between the initial and final prolonged QTc appeared, in addition to 16 cases of prolonged QTc syndrome.

In nine individuals, unifascicular blocks also disappeared, three were cases of first degree A-V block, three were arrhythmias, four were ventricular arrhythmias (extrasystoles), five were supraventricular arrhythmias, and one suggested ventricular hypertrophy. In 27 cases (48.2%), of which five were supraventricular arrhythmias, four were ventricular arrhythmias (extrasystoles), three were cases of first degree A-V block, three were arrhythmias, four were ventricular arrhythmias (extrasystoles), five were supraventricular arrhythmias, and one suggested ventricular hypertrophy.

The difference between the initial and final prolonged QTc was statistically significant (P < 0.01, by Fisher's exact test).

Table 3 shows the evolution of the ECG in the 53 cases whose tracings were normal at the beginning of the trial but who presented alterations one year later. After treatment with itraconazole or allopurinol, the ECG tracings reverted to normal in 27 cases (48.2%), of which five were supraventricular arrhythmias, four were ventricular arrhythmias (extrasystoles), three were cases of first degree A-V block, three were cases of ischemia, and one suggested ventricular hypertrophy. In nine individuals, unifascicular blocks also disappeared, in addition to 16 cases of prolonged QTc syndrome. The difference between the initial and final prolonged QTc was statistically significant (P < 0.01, by Fisher's exact test).

Of the Chagas' disease patients with cardiopathy treated with itraconazole and allopurinol, whose tracings were normal at the beginning of the trial but who presented alterations one year later. After treatment with itraconazole or allopurinol, 53% and 44%, respectively, were parasitologically cured. The xenodiagnosis results for patients receiving itraconazole or allopurinol were both significantly different compared with placebo treatment (P < 0.001, by Fisher's exact test).

Table 4 shows the evolution of the ECG in 103 Chagas' disease patients with cardiopathy treated with itraconazole or allopurinol. Of the 110 who received itraconazole and the 127 who received placebo did not demonstrate parasitologic cure. The other cases considered to be treatment failures correspond to individuals whose xenodiagnosis results were negative before and after treatment in whom parasitologic cure was difficult to gauge.

Of the Chagas' disease patients with cardiopathy whose ECG remained altered during the four-year follow-up, 31% of those who received itraconazole and 14.3% who received allopurinol showed parasitologic cure. In contrast, 34.5% of those who received itraconazole and 10.7% of those who received allopurinol did not demonstrate parasitologic cure. The other cases had negative xenodiagnosis results and CoML results both before and after treatment.

In the indeterminate group, of the 129 persons who received placebo, 1.6% showed subsequent parasitologic cure. Of the 110 who received itraconazole and the 127 who received allopurinol, 53% and 44%, respectively, were parasitologically cured. The difference between placebo and itraconazole and placebo and allopurinol was statistically significant (P < 0.001). No statistically significant differences were found between itraconazole and allopurinol.

**DISCUSSION**

In acute Chagas' disease, the criterion for cure is clinical and parasitologic improvement detected by xenodiagnosis, hemoculture, and negative serologic test results. However, in chronic Chagas' disease, there is no clear standard for cure. There may be clinical and ECG improvement or negative xenodiagnosis results, but neither of these alone is a sure sign of cure. Conventional serology alone is also of little help because it shows positive results for many years. However, improvement of all of these indicators, including CoML, and of clinical parameters over several years can indicate cure.

After four years of follow-up in this study, of 43 subjects with positive xenodiagnosis results who were treated with itraconazole, the xenodiagnosis result became negative in 38 (88.4%). The same occurred in 18 (62%) of the 29 xenopositive-positive patients who received allopurinol, but in only 25% of xenopositive-positive patients who received placebo. Previous experience in Chile indicates that under optimal conditions the sensitivity of xenodiagnosis is approximately 70% in chronic infections, so we expected no more than 30% of the xenodiagnosis results to become negative. The xenodiagnosis results for patients receiving itraconazole or allopurinol were both significantly different compared with placebo treatment (P < 0.05), and we noted that the majority of patients whose xenodiagnosis results remained positive during follow-up had received placebo. Of those patients with negative xenodiagnosis results prior to treatment, the test result became positive in seven in the placebo group, in two of 111 patients treated with itraconazole (1.8%), and in four of 153 patients treated with allopurinol (2.6%). These are treatment failures.

In contrast to the xenodiagnosis results, all conventional serologic test results remained positive during the four years of follow-up, except for 14 cases in which the IHA titers decreased. This confirms the idea that conventional serology is unsatisfactory as a criterion for cure.

Both itraconazole and allopurinol led to improvements in ECG evaluations. The ECG reverted from abnormal to normal in 39%, although some of these changes could have other explanations. Arrhythmias, ischemic changes, and prolonged QTc can be intermittent and require long-term study. However, since alteration of intraventricular conduction could only be caused by lesions of the conductive syst-
of patients with chronic Chagas' disease.

Of those patients in the indeterminate group whose ECG became abnormal during the first year of follow-up and who were treated, the alterations returned to normal in 48.2%. The alterations that appeared in their tracings suggest that the lesions had relatively short evolution, in contrast to those of the chronic cardiopathies mentioned earlier. This reinforces the need for early detection and treatment of patients with Chagas' disease.

For the ECG evaluations, we applied criteria established in our previous follow-up work with Chagas' disease patients with cardiopathy. Alterations such as prolonged QTc and incomplete right bundle branch block in children and young adults were interpreted as representing the initial manifestations of heart involvement. During the evolution of chronic cardiopathy, there are periods of transient normalization of the ECG, although in more advanced stages the changes tend to become irreversible. This evolution of the ECG is related to histopathologic alterations of the myocardium. The ECG changes in Chagas' disease are an active process that may improve spontaneously, as has been shown in experimentally infected mice and dogs. Our results confirm that individuals with indeterminate ECGs that shift spontaneously from normal to pathologic can return to normal with treatment. These ECG tracings can then remain normal for at least four years. These findings represent a modification of the natural history of Chagas' cardiopathy. The annual rate of new Chagas' disease patients with cardiopathy among treated patients is much less than the 9% that would be expected during the evolution of untreated disease.

In this study, we chose to administer allopurinol treatment for two months because other investigators have used it in this manner with good results against chronic Chagas' disease. We were also cautious about possible side effects with allopurinol at a dosage of 600 mg/day for more than two months. In contrast, itraconazole is normally used for the treatment of systemic mycotic infections at a dosage of 400 mg/day for more than four months without significant side effects.

Both drugs were well tolerated. Only one patient taking itraconazole had to discontinue the medication because of side effects. High cure rates were achieved with both drugs. Eighty-eight percent of the patients who began the study with a positive xenodiagnosis result for Chagas' disease were cleared by itraconazole while 66% were cleared by allopurinol. Sixteen patients who began the study with a negative xenodiagnosis result became CoML-negative during this manner with good results against chronic Chagas' disease. We were also cautious about possible side effects with allopurinol at a dosage of 600 mg/day for more than two months. In contrast, itraconazole is normally used for the treatment of systemic mycotic infections at a dosage of 400 mg/day for more than four months without significant side effects.

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