Treatment of Chagas’ disease with itraconazole: electrocardiographic and parasitological conditions after 20 years of follow-up

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Objectives: To evaluate cases of chronic Chagas’ disease for the long-term effects of treatment with itraconazole on Trypanosoma cruzi infections and the regression or development of ECG abnormalities.

Methods: In March 1992, we treated 46 patients with chronic Chagas’ disease with 6 mg/kg/day of itraconazole for 120 days in a blind evaluation. The patients came from an area of Chile where the disease was endemic and were checked for ECG abnormalities and with xenodiagnosis (XD) or real-time XD-quantitative PCR (XD-qPCR) for Trypanosoma cruzi infection before treatment and once a year for 20 years.

Results: Twenty-one patients proved to be uninfected after 20 years and 15 of the patients had a normal ECG. Of the latter cases, 32.6% could be considered cured, although all of them had positive serology. Itraconazole prevents the development of ECG abnormalities, because after 20 years of treatment only 10.86% of patients developed ECG abnormalities (Z = 1.70, P = 0.046). XD-qPCR performed on 16 patients demonstrated 10 cases with <1.42 parasites/mL: eight with <1 parasite/mL, one with 1.42 parasites/mL and one with 1.01 parasites/mL. Five patients had more than 11.75 parasites/mL, all of them with a positive XD; these cases correspond to therapy failure, since re-infection was ruled out. In one case, XD-qPCR did not present amplification.

Conclusions: Itraconazole is useful in the treatment of chronic Chagas’ disease as it prevented the development of ECG abnormalities and cured 32.6% of patients.

Keywords: electrocardiographic abnormalities, triazole drugs, posaconazole, ravuconazole, nifurtimox

Introduction

American trypanosomiasis is a neglected tropical disease caused by Trypanosoma cruzi, which is endemic from Patagonia to the northern border of Mexico.1 An important flow of migration from Latin America to the northern countries at the beginning of the 21st century caused the emergence of imported cases in non-endemic countries such as the USA, Australia, Europe and Japan. It is estimated that there are 350000 cases in the USA and 100000 cases in Europe, 87000 of which are in Spain.2

There are 8–10 million individuals infected with T. cruzi in Latin America, more than half of whom are in the Southern Cone. Uruguay, Chile, Brazil, Paraguay, part of Argentina, Peru and Bolivia have been declared free of transmission by Triatoma infestans, the main vector of the disease.3 In Chile, 150000 persons are infected with T. cruzi; 30% of them will develop some degree of cardiopathy and one in three will probably require a pacemaker to survive.4,5

At present, there is a consensus that all the individuals who are infected with T. cruzi should be treated unless they have reached the late chronic phase of the infection or have developed decompensated chronic heart failure.6

No drug approved for clinical use currently provides entirely satisfactory treatment for chronic T. cruzi infection although there are many drugs with activity against T. cruzi; the only ones that can currently be given to humans on the basis of ethical and clinical considerations are nifurtimox and benznidazole. Unfortunately, the use of these drugs has been limited by their restricted availability and the significant adverse effects that they sometimes cause in adults.7,8

An efficient, safe, widely available and low-cost drug for the treatment of chronic Chagas’ disease is required. For this

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purpose, two antimycotics—posaconazole (Merck Sharp & Dohme)\(^\text{11}\) and ravuconazole (Bristol Myers Squibb),\(^\text{12}\) drugs with good efficacy against \(T. \) cruzi—have been applied to chronic chagasic patients (in Phase II protocols). Itraconazole, a synthetic imidazole derivative similar to posaconazole,\(^\text{13,14}\) has shown good efficacy against \(T. \) cruzi both \emph{in vitro} and \emph{in vivo}, resulting for example in the total cure of chronically infected mice.\(^\text{15,16}\) This drug has been used as an efficient antimycotic in humans for the last 32 years without causing important side effects. The aim of this study was to evaluate, among cases of chronic Chagas’ disease, the long-term effects of treatment with itraconazole on \(T. \) cruzi infections and the regression or development of ECG abnormalities.

### Methods

#### Patients

The present study represented a 20 year follow-up of patients treated with itraconazole 6 mg/kg/day for 120 days in a blind evaluation of the efficacy of the drug in the treatment of chronic American trypanosomiasis.\(^\text{17}\) This investigation was accepted by the Ethics Committee of the Faculty of Medicine of the University of Chile and informed consent was given by all patients (Project Fondecyt 092/0920). A total of 46 patients were checked for ECG abnormalities and microscopic or molecular evidence of current \(T. \) cruzi infections. Each patient was subjected to a 12-lead ECG examination before treatment, after 4, 8 and 12 months and once a year for 19 years, following the blind protocol recommended by the WHO.\(^\text{18}\) The investigator analysing the ECG traces was unaware of each patient’s infection status in the previous follow-up and/or for any follow-up.

In the formal analysis, the results of the ECG examinations from the 20 year follow-up were compared with the corresponding results recorded earlier. This allowed each patient to be categorized as: normal/normal (indicating prevention of cardiopathy), these cases having a normal ECG at baseline and during the yearly check-ups until the 20 year follow-up; abnormal/normal (indicating regression of the cardiopathy); these patients having an abnormal ECG at baseline and during the first check-ups, but a normal ECG in the final 5 years; normal/abnormal (indicating conversion), these cases having a normal ECG at baseline, during the serial follow-up and in the last 5 years developed an abnormal ECG; and abnormal/abnormal (indicating maintenance of the cardiopathy), these patients having a abnormal ECG at baseline and during the yearly check-ups until the 20 year follow-up.\(^\text{19,20}\) The abnormal ECG traces were classified as major and minor. Major abnormalities were conduction alterations such as second- and third-degree atrioventricular block or intraventricular blocks, ischaemia on imaging and a prolonged QTc interval. Minor abnormalities were rhythm alterations such as auricular tachycardia, extrasystoles, auricular parasyctoles, ventricular parasyctoles and short runs of ventricular tachycardia.

#### Control group

Sixty-seven patients with chronic Chagas’ disease of indeterminate duration were followed for 4 years; these patients constituted the historical control group (1981 – 84). For ethical reasons, this group was treated after the experimental period.

### Parasitology

Every 12 months for 20 years, a 2 mL sample of venous blood was collected from each patient, and in a xenodiagnosis (XD) method uninfected \(T. \) infestans were allowed to feed on the patients. Both procedures were performed simultaneously.\(^\text{13}\) PCR assays were then used to check the blood sample, and XD-quantitative PCR (XD-qPCR) was applied to faecal samples (FS) to check for \(T. \) cruzi DNA in a random sample of XD applied to 16 patients. Each test was duplicated. All the FS of \(T. \) infestans were also studied under the microscope as wet smears for flagellates. A patient was positive if he or she was considered to be infected with \(T. \) cruzi on any of these tests.

#### Xenodiagnosis

Seven third- or fourth-instar nymphs of \(T. \) infestans that were free of infection and held in a mesh-topped wooden box were fed on a patient and then maintained at 27°C and 80% humidity. Microscopic examination of the FS was performed 30, 60 and 90 days after feeding the triatomines. For XD-qPCR, the FS of all the triatomines obtained after each period of incubation were mixed with 500 μL of PBS buffer at pH 7.2, incubated at 98°C for 15 min and then centrifuged at 4000 g for 3 min. The supernatants were stored at −20°C until the DNA was extracted for use.\(^\text{21}\)

#### DNA extraction from blood

A sub-sample (2 mL) of each patient’s blood was mixed with the same volume of a 6 M guanidine hydrochloride/0.2 M EDTA solution, incubated at 98°C for 15 min to break the minicircles of \(T. \) cruzi kinetoplasts and stored at 4°C. DNA extraction was performed on 200 μL of guanidine-mixed samples, using the FavorPrep Blood Genomic DNA Extraction Kit (Favorgen Biotech Corp) according to the manufacturer’s instructions, and maintained at −20°C until use.\(^\text{21}\)

#### PCR of blood

The PCR assay used to investigate the blood DNA samples was performed in triplicate with oligonucleotides 121 and 122, which anneal to the four constant regions present in minicircles of \(T. \) cruzi, to obtain 320 bp amplification.\(^\text{19,21}\) PCR was performed in a final volume of 20 μL containing 2.5 mM MgCl\(_2\), 0.2 mM of each dNTP, 0.5 μM of each primer (121 and 122) and 1 unit of GoTaq DNA polymerase (Promega Corp). The amplification programme was performed in a TC-412 thermal cycler (Techne), and included an initial denaturation at 98°C for 1 min and 64°C for 2 min, 33 cycles of 94°C for 1 min, 64°C for 1 minute and 72°C for 1 min, and a final elongation at 72°C for 10 min. Each experiment included 5 μL of BenchTop 100 bp DNA ladder (Promega Corp), a PCR control that contained water instead of DNA, DNA from non-chagasic patients and as a positive control the purified DNA-\(T. \) cruzi Tulahuen strain. Amplification products were analysed by electrophoresis in a 2% agarose gel and visualized by staining with GelRed (Biotium).\(^\text{21}\) The analytical sensitivity was determined by qPCR of blood using 50 chronic chagasic cases with demonstrated parasitaemia by XD and PCR in the blood. The lower limit of detection was 0.1 parasites/mL (I. Zulantay, E. Araya, M. Soavedra, W. APT, G. Martinez, unpublished results).

#### DNA extraction from FS

Before the FS-DNA isolation process, 20 ng equivalent of negative human blood DNA was added as an exogenous internal control.\(^\text{23}\) DNA purification was performed using a 100 μL initial volume with the FavorPrep Blood Genomic DNA Extraction Mini Kit (Favorgen, Biotech Corp.), modified by omitting the step of cell lysis with proteinase K. The elution of the FS was performed 30, 60 and 90 days after feeding the triatomines. A patient was positive if he or she was considered to be infected with \(T. \) cruzi on any of these tests.

#### XD-qPCR

qPCR was applied to 16 patients in FS obtained by XD (XD-qPCR) in the 20 year control to determine the presence and amount of \(T. \) cruzi,
using the TaqMan detection system in a thermocycler (Stratagene MXP3000P, Agilent Technologies, Inc.) under conditions suggested by the manufacturer and using Cruzi 1 satellite (5′-ASTCGGTGTTTCTGGA-3′), Cruzi 2 (5′-AATCTCCTCAACGCGGATA-3′) DNA primers and the DNA satellite probe Cruzi 3 (5′-CACACGTCGTACCCA-3′). The reaction mixture consisted of 2 μL of the sample to be investigated, 10 μL of Brilliant Multiplex QPCR Master Mix (Stratagene, Agilent Technologies), 0.5 μL of a 1:500 dilution of a reference dye (ROX), 1 μL of each of the oligonucleotides Cruzi 1 and Cruzi 2, 0.4 μL of the Cruzi 3 probe, 0.2 μL of BSA (100x) (BioLabs) and 4.9 μL of molecular biology grade water (Mo Bio Laboratories, Inc.), in a final reaction volume of 20 μL. To obtain a standard curve to perform the quantification, we used a stock of epimastigote forms of T. cruzi Tulahuen strain, starting with a known concentration of parasite DNA and performing serial dilutions (1:10) defining seven points ranging from 10^5 to 10^−4 parasites/mL. The sensitivities of each parasitological test were compared and the relationship between the infection status and ECG developments observed were explored statistically using the χ² test, Fisher’s exact test, McNemar test and percentage differences, considering P values <0.05 as significant.

Results
By comparing the pre-treatment ECG with those performed during the 20 follow-up years, the 46 patients investigated in this study were categorized as normal/normal, abnormal/normal, normal/abnormal or abnormal/abnormal. These groups were similar in mean age and in the prevalence of infection, as indicated by positivity of any one of the parasitological tests 20 years post-treatment. Twenty-one (45.6%) of the 46 patients studied were infected. Of the patients with a normal ECG at baseline who maintained the ECG tracing at 20 years, 34.7% were infected. No statistical differences were observed between the ECG results and the infection. One case with an abnormal pre-treatment ECG tracing, left anterior hemiblock and incomplete right bundle branch block had normal traces after 20 years of follow-up. Five patients with a normal pre-treatment ECG had abnormal traces at least 20 years after treatment; two had a prolonged QTc interval, one had a prolonged QTc and sinus bradycardia, one had sinus bradycardia and one had left hypertrophy (Table 1).

Table 1. The results of the ECG investigations and parasitological study 20 years post-treatment of 46 patients treated with itraconazole

<table>
<thead>
<tr>
<th>ECG at 20 years</th>
<th>Found infected</th>
<th>Found uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>23</td>
<td>50</td>
</tr>
<tr>
<td>Abnormal</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Normal</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Abnormal</td>
<td>17</td>
<td>37</td>
</tr>
</tbody>
</table>

No association was found between the ECG results and T. cruzi infection. Fisher’s exact P value = 0.375.

Table 2. Patients treated with itraconazole found to have normal or abnormal ECG traces pre-treatment and 20 years post-treatment and the results of XD and T. cruzi PCR performed 20 years post-treatment

<table>
<thead>
<tr>
<th>ECG pre-treatment</th>
<th>XD</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 20 years</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>(+)</td>
</tr>
<tr>
<td>Normal</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>Abnormal</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Normal</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Abnormal</td>
<td>17</td>
<td>5</td>
</tr>
</tbody>
</table>

There is no association between the results of XD and PCR in the normal/normal group. Fisher’s exact test, P = 0.035.

There was not enough information in one case. No association was found between the results of XD and PCR in the normal/abnormal and abnormal/abnormal groups. Fisher’s exact test, P = 0.5 and 0.296, respectively.

No association was found between the results of ECG and XD and the results of PCR in the pre-treatment period. Fisher’s exact test, P = 0.157 and χ² = 0.708, respectively.

No association was found between the results of the final ECG, XD and PCR. χ² = 2.571788, P = 0.113; χ² = 0.763, P = 0.382, respectively.

patients with a normal pre-treatment ECG had abnormal traces at least 20 years after treatment; two had a prolonged QTc interval, one had a prolonged QTc and sinus bradycardia, one had sinus bradycardia and one had left hypertrophy (Table 1).

Only 10 patients were positive for T. cruzi by XD (21.7%), but 21 had a positive PCR for a blood sample (45.7%). XD was significantly less likely to give a positive result for a patient than was the PCR-based assay of a blood sample from the sample patient. Positive XD had the same results with PCR, nevertheless, 30.5% of the negative XD had positive PCR (McNemar test P < 0.001; Table 2).

Most of the patients with major ECG abnormalities maintained the alterations after 20 years (Table 3). The patients with minor abnormalities (for example extrasystoles and parasystoles) showed an improvement in these alterations 20 years after treatment (Table 3).

The XD-qPCR performed on 16 treated patients demonstrated very low parasite loads. Ten had <1.42 parasites/mL, eight of
Abnormality | Pre-treatment | 20 years post-treatment
--- | --- | ---
Unifascicular blocks | | |
left anterior hemiblock | 2 | 1 |
left anterior hemiblock and prolonged QTc interval | 3 | 3 |
Left anterior hemiblock and ischaemia | — | 1 |
Incomplete right bundle branch block | — | 1 |
Incomplete right bundle branch block and prolonged QTc interval | — | 1 |
Incomplete right bundle branch block and left anterior hemiblock | — | 1 |
Complete right bundle branch block and left anterior hemiblock | 3 | 3 |
Complete right bundle branch block and left posterior hemiblock | 2 | 2 |
Complete right bundle branch block | 2 | 2 |
Left ventricular hypertrophy | 1 | 1 |
Prolonged QTc interval | 4 | 1 |

Table 3. The ECG abnormalities in the 17 patients categorized as abnormal/abnormal (abnormal ECG traces pre-treatment and 20 years post-treatment with itraconazole)

Table 4. Results of XD-qPCR for Trypanosoma cruzi applied to 16 treated patients with chronic Chagas' disease

<table>
<thead>
<tr>
<th>Number</th>
<th>Parasites/mL</th>
<th>T. cruzi Ct</th>
<th>X12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.01</td>
<td>35.87</td>
<td>30.48</td>
</tr>
<tr>
<td>2</td>
<td>(&lt;) not quantifiable</td>
<td>38.84</td>
<td>32.05</td>
</tr>
<tr>
<td>3</td>
<td>&lt;1</td>
<td>34.79</td>
<td>32.88</td>
</tr>
<tr>
<td>4</td>
<td>56.68</td>
<td>27.92</td>
<td>33.79</td>
</tr>
<tr>
<td>5</td>
<td>&lt;1</td>
<td>34.87</td>
<td>32.97</td>
</tr>
<tr>
<td>6</td>
<td>&lt;1</td>
<td>35.32</td>
<td>32.69</td>
</tr>
<tr>
<td>7</td>
<td>&lt;1</td>
<td>34.87</td>
<td>32.93</td>
</tr>
<tr>
<td>8</td>
<td>1.42</td>
<td>32.18</td>
<td>32.19</td>
</tr>
<tr>
<td>9</td>
<td>&lt;1</td>
<td>34.3</td>
<td>30.93</td>
</tr>
<tr>
<td>10</td>
<td>217</td>
<td>26.39</td>
<td>31.98</td>
</tr>
<tr>
<td>11</td>
<td>95.46</td>
<td>27.64</td>
<td>30.51</td>
</tr>
<tr>
<td>12</td>
<td>&lt;1</td>
<td>No Ct</td>
<td>30.81</td>
</tr>
<tr>
<td>13</td>
<td>(-)</td>
<td>No Ct</td>
<td>30.81</td>
</tr>
<tr>
<td>14</td>
<td>&lt;1</td>
<td>35.64</td>
<td>31.7</td>
</tr>
<tr>
<td>15</td>
<td>11.75</td>
<td>29.88</td>
<td>31.8</td>
</tr>
<tr>
<td>16</td>
<td>422.7</td>
<td>25.43</td>
<td>31.24</td>
</tr>
<tr>
<td>17 FAM negative control</td>
<td>No Ct</td>
<td>No Ct</td>
<td></td>
</tr>
<tr>
<td>18 Buffer</td>
<td>No Ct</td>
<td>No Ct</td>
<td></td>
</tr>
<tr>
<td>19 NTC</td>
<td>No Ct</td>
<td>No Ct</td>
<td></td>
</tr>
<tr>
<td>20 FAM positive control</td>
<td>27.28</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>21 FAM positive control X12</td>
<td>—</td>
<td>—</td>
<td>31.8</td>
</tr>
</tbody>
</table>

In one case (6.25%), the sample of XD of patients did not present an amplification (<). In one case (6.25%) it was not possible to quantify the parasite number (<= not quantifiable) as the amount of parasite was lower than the dynamic detection range of the technique, that is, 0.1 parasites/mL.

FAM, 6-carboxifluorescein; No Ct, no threshold cycle; NTC, no template control.

Discussion

The patients categorized as normal/abnormal in the last or earlier follow-ups were initially chosen as part of the infected historical control group. By 20 years post-treatment, however, 10.9% of those who had had a normal ECG pre-treatment had developed detectable ECG abnormalities, most of which were minor ECG alterations. Those found to have developed ECG alterations between the initiation of treatment and the 20 year follow-up still had abnormal ECG traces almost two decades later (Table 3). The one patient with an abnormal ECG at baseline who showed normal ECG traces after treatment and follow-up indicates that itraconazole can cure some T. cruzi-related cardiopathy (Table 1). Of the patients who had normal ECG traces at baseline—28 of 46 (60.8%)—only five showed evidence of ECG abnormalities during the 20 years of follow-up, which represents an annual rate of 0.5%; this indicates that itraconazole they received stopped, or at least slowed, the natural evolution of abnormalities in their ECG traces. This lower annual rate of ECG abnormalities was statistically significant (Z = 2.03, P = 0.0212).

During the 20 years of follow-up, no mortality attributable to T. cruzi infection was observed in the treated patients. This is encouraging and could indicate some effect of the treatment, since there is a 1.85% annual risk of death among patients with Chagas cardiopathy who are left untreated.4

The results of XD-qPCR, performed in 16 patients, confirm the effect of itraconazole on T. cruzi load after 20 years of treatment, since 10 of the cases had a parasite load between <1 (eight cases) to 1.42 parasites/mL (one 1.01 and the other 1.42). In one case, no amplification of the XD sample could be obtained. In five cases, the parasite load detected was between 11.75 and 422.7 parasites/mL; all these cases had a positive XD. The results of these cases clearly demonstrated treatment failure because reinfection is unlikely due to control of T. infestans.25

The number of parasites quantified by XD-qPCR could be related to the efficiency of replication in the insect and the initial inoculum during feeding. However, this hypothesis must be confirmed by other investigations.

Twenty-five (54.3%) of the patients investigated were negative in all the parasitological tests used, and 15 (60%) of these cases had normal ECG traces at 20 years follow-up. These parasite-negative individuals with a normal ECG should probably be considered ‘cured’ but all of them still appeared to be sero-positive at 20 years follow-up, with titres ranging between 1/20 and 1/160 for indirect immunofluorescence and an optical
density of 0.1–1.2 with ELISA. The persistence of T. cruzi antigens in central memory T cells in the absence of infection was described by Bustamante et al. in 2008; this could explain the persistent seropositivity of the treated patients of this study in the absence of T. cruzi infection.

The present results indicate that itraconazole is effective in the treatment of chronic American trypanosomiasis in Chile. Most of the circulating strains in the area where Chagas’ disease is endemic correspond to TcII, which is sensitive to itraconazole. It is possible that the cases that did not respond to treatment had been infected with strain TcI, which is resistant to this drug.

No drug currently used to treat chronic chagasic patients has a high efficacy and low number of secondary effects. Itraconazole is an azole drug that has demonstrated few side effects (one case of hepatic toxicity has been described) in relation to other drugs currently used, such as nifurtimox or benznidazole, and can prevent the development of ECG abnormalities as shown here, and cause the regression of some ECG abnormalities. Posaconazole and ravuconazole, other azoles that belong to the triazole family, have demonstrated high efficiency in in vitro and in vivo experiments. Current international Phase II clinical studies involving these drugs will allow a determination of their efficacy. We hope that there will be in the near future an integrated solution for individuals infected with T. cruzi, who represent an important public health problem.

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**Transparency declarations**

None to declare.

**References**


