J Antimicrob Chemother 2013; **68**: 2164–2169
doi:10.1093/jac/dkt135 Advance Access publication 3 May 2013

Journal of Antimicrobial Chemotherapy

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

Werner Apt[1]*, Arturo Arribada[2], Inés Zulantay[1], Jorge Rodríguez[3], Miguel Saavedra[1] and Andrea Muñoz[1]

[1]Laboratorio Parasitología Básico Clínico, Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Independencia 1027, PO Box 427, Santiago 3, Chile; [2]Servicio de Cardiología, Clínica INDISA, PO Box 9183, Santiago 1, Chile; [3]Escuela de Salud Pública, Facultad de Medicina, Universidad de Chile, PO Box 9183, Santiago 1, Chile

*Corresponding author. Laboratorio Parasitología Básico Clínico, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, PO 427 Santiago 3, Chile. Tel: +56-2-9786122; Fax: +56-2-9786122; E-mail: wapt@med.uchile.cl

Received 22 November 2012; returned 21 January 2013; revised 29 January 2013; accepted 22 March 2013

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.[7–10]

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

purpose, two antimycotics—posaconazole (Merck Sharp & Dohme)[11] and ravuconazole (Bristol Myers Squibb),[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,[13,14] has shown good efficacy against *T. cruzi* both *in vitro* and *in vivo*, resulting for example in the total cure of chronically infected mice.[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.[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.[4,18] 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.[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 parasystoles, ventricular parasystoles 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.[21] 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.[21]

DNA extraction from blood

A subsample (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.[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 amplicons.[22] 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 min 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* Tulahuén strain. Amplification products were analysed by electrophoresis in a 2% agarose gel and visualized by staining with GelRed (Biotium).[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. Saavedra, W. Apt, G. Martínez, 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.[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 process was similar to that described for human blood. Samples were maintained at −20°C until XD-qPCR was carried out.

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′-ASTCGG CTGATCGTTTTCGA-3′), Cruzi 2 (5′-AATTCCTCCAAGCAGCGGATA-3′) DNA primers and the DNA satellite probe Cruzi 3 (5′-CACACACTGGACACCAA-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 satellite oligonucleotides Cruzi 1 and Cruzi 2, 0.4 μL of the Cruzi 3 probe, 0.2 μL of BSA (100×) (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* Tulahuén strain, starting with a known concentration of parasite DNA and performing serial dilutions (1:10) defining seven points ranging from 10^5 to 10^{-1} parasites/mL (R^2 0.99, Y −3.383, efficiency 97.1%) (M. Saavedra, E. Araya, I. Zulantay, W. Apt, unpublished results).

All the calculations were made assuming that *T. cruzi* genetic material has a mass of 200 fg.[24] In parallel, as an internal exogenous control, human chromosome 12 (X12) was used, adding a quantity equivalent to 20 ng of human genomic DNA free of the disease to each of the FS before the extraction process. The exogenous internal control was designed, first, to discard false-negative cases caused by the absence of DNA in the evaluated sample due to extraction problems, and second, to assess whether there was inhibition in the PCR reaction. The primers N1X12 forward (5′-AGCTGGCTAGACTGTCAT-3′) and N2X12 reverse (5′ CTTTGCCGTTGAAGCTTG-3′) and the probe N3X12 (not published) were used. The reaction mixture was composed of 2 μL of the sample to be investigated, 10 μL of Brilliant Multiplex QPCR Master Mix, 0.5 μL of a 1:500 solution of ROX, 2 μL of N3X12, 0.2 μL of BSA (100×) and 2.5 μL of molecular biology grade water, in a final reaction volume of 20 μL. To produce a standard curve, a 1:5 dilution of human genomic DNA was used with a known amount of X12, utilizing six known points to quantify the sample.[21] Both reactions were performed in the same assay with three respective controls, using the same thermal profile, which consisted of 10 min of pre-incubation at 95°C and 40 cycles of amplification. The emitted fluorescence recording was performed at 60°C at the end of each cycle. Results were analysed with the software MxPro X4.1 (Agilent Technologies).

Statistics

The sensitivities of each parasitological test were compared and the relationship between the infection status and ECG developments observed were explored statistically using the χ^2 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

				Found infected		Found uninfected	
Pre-treatment	ECG at 20 years	*n*	%	*n*	%	*n*	%
Normal	normal	23	50	8	34.7	15	65.2
Abnormal	normal	1	2	1	100	0	0
Normal	abnormal	5	11	3	60.0	2	40.0
Abnormal	abnormal	17	37	9	52.9	8	47.0

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

			XD		PCR	
ECG pre-treatment	At 20 years	*n*	(+)	(−)	(+)	(−)
Normal	normal	23	2	21	8	15
Abnormal	normal	1	1	0	1	0
Normal	abnormal	5	2	3	3	2
Abnormal	abnormal	17	5	12	9	8

There is an 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 $\chi^2=0.708$, respectively.

No association was found between the results of the final ECG, XD and PCR. $\chi^2=2.57178$, $P=0.113$; χ^2 0.763, $P=0.382$, respectively.

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

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)

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

Number	Parasites/mL	Ct	
		T. cruzi	X12
1	1.01	35.87	30.48
2	(+) not quantifiable	38.84	32.05
3	<1	34.79	32.88
4	56.68	27.92	33.79
5	<1	34.87	32.97
6	<1	35.32	32.69
7	<1	34.87	32.93
8	1.42	32.18	32.19
9	<1	34.3	30.93
10	217	26.39	31.98
11	95.46	27.64	30.51
12	<1	33.97	30.82
13	(−)	No Ct	30.81
14	<1	35.64	31.7
15	11.75	29.88	31.8
16	422.7	25.43	31.24
17 FAM negative control		No Ct	No Ct
18 Buffer		No Ct	No Ct
19 NTC		No Ct	No Ct
20 FAM positive control		27.28	—
21 FAM positive control X12		—	31.8

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.

them with <1 parasite/mL, one with 1.42 parasite/mL and one with 1.01 parasites/mL. In one case, the patient's XD sample did not show amplification. Only five cases had more than 11.75 parasites/mL, all of which had a positive XD (Table 4).

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 the itraconazole they received stopped, or at least slowed, the natural evolution of their trypanosomiasis (Tables 1 and 2) since in a historical control group with Chagas' disease in Chile followed for 4 years, we demonstrated that 9.7% per year of the patients with a normal ECG (indeterminate period) developed abnormalities in their ECG traces.[4] 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 result 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 seropositive 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;[26] 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.[27] It is possible that the cases that did not respond to treatment had been infected with strain TcI, which is resistant to this drug.[28]

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)[15,19,29] in relation to other drugs currently used, such as nifurtimox or benznidazole,[30,31] 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.

Funding

This work was supported by FONDECYT Projects 1120382 and 1100768.

Transparency declarations

None to declare.

References

1 Lescure F, Le Loup G, Freilij H *et al*. Chagas disease: changes in knowledge and management. *Lancet Infect Dis* 2010; **10**: 556–70.

2 Le Loup G, Pialoux G, Lescure F *et al*. Update in treatment of Chagas disease. *Curr Opin Infect Dis* 2011; **24**: 428–34.

3 Salvatella R. Control de la enfermedad de Chagas en las sub-regiones de América. XXV Reunión Científica Anual de la Sociedad Argentina de Protozoología y Enfermedades Parasitarias. *Rev Arg Sal Publ* 2012; numero especial. pp: 13–4.

4 Arribada A, Apt W, Ugarte J. A four year follow up survey of chagasic cardiopathy in Chile. *Bull Pan Am Health Organ* 1986; **20**: 254–66.

5 Prata A. Clinical and epidemiological aspect of Chagas disease. *Lancet Infect Dis* 2001; **1**: 92–100.

6 Apt W. Current and developing therapeutic agents in the treatment of Chagas disease. *Drug Des Devel Ther* 2010; **4**: 243–53.

7 Bern C. Antitrypanosomal therapy for chronic Chagas' disease. *N Engl J Med* 2011; **364**: 2527–34.

8 Murcia L, Carrilero B, Albajar Viñas P *et al*. Nifurtimox chemotherapy: collateral effects in treated *Trypanosoma cruzi* infected patients. *Rev Esp Quimioter* 2012; **25**: 74–5.

9 Valencia C, Mancilla M, Ramos D *et al*. Tratamiento de la enfermedad de Chagas crónica en Chile. Efectos adversos de nifurtimox. *Rev Ibero-Latinoam Parasitol* 2012; **71**: 97–108.

10 Altcheh J. Estudios farmacológicos de benznidazol y nifurtimox en niños y mujeres lactantes con enfermedad de Chagas. In: *VIII Taller sobre la enfermedad de Chagas importada. Avances en el tratamiento antiparasitario*. Barcelona: Institute for Global Health Ed., 2012; 40–4.

11 Morrillo C. Actualización estudio clínico Stop-Chagas [Study of oral posaconazole in the treatment of asymptomatic chronic Chagas disease]. In: *VIII Taller sobre la enfermedad de Chagas importada*. Barcelona: Institute for Global Health Ed., 2012; 17–20.

12 Torrico F, Ribeiro I, Alonso-Vega C *et al*. Justificación y diseño de un estudio Fase II de prueba de concepto del E1224, un nuevo fármaco candidato para el tratamiento de pacientes adultos con enfermedad de Chagas crónica. In: *VIII Taller sobre la enfermedad de Chagas importada*. Barcelona: Institute for Global Health Ed., 2012; 22–4.

13 Urbina J. Specific chemotherapy of Chagas disease: relevance, current limitations and new approaches. *Acta Trop* 2010; **115**: 55–68.

14 Urbina J, Payares G, Contreras L *et al*. Antiproliferative effect and mechanism of action of SOH56592 against *Trypanosoma* (Schizotrypanum) *cruzi*. In vitro and in vivo studies. *Antimicrob Agents Chemother* 1998; **42**: 1771–7.

15 McCabe E, Remington J, Araujo F. In vitro and in vivo effect of itraconazole against *Trypanosoma cruzi*. *Am J Trop Med and Hyg* 1986; **35**: 280–4.

16 Marr J. Purine analogs as chemotherapeutic agents in leishmaniasis and American trypanosomiasis. *J Lab Clin Med* 1991; **118**: 111–9.

17 Apt W, Aguilera X, Arribada A *et al*. Treatment of chronic Chagas' disease with itraconazole and allopurinol. *Am J Trop Med Hyg* 1998; **59**: 133–8.

18 Maguire J, Mott K, Souza J *et al*. Clasificación de electrocardiogramas y sistema abreviado de derivaciones para encuestas de poblaciones en relación con la enfermedad de Chagas. *Bol Of San Panam* 1982; **93**: 102–17.

19 Apt W, Arribada A, Zulantay I *et al*. Itraconazole or allopurinol in the treatment of chronic American trypanosomiasis: the regression and prevention of electrocardiographic abnormalities during 9 years of follow-up. *Ann Trop Med Parasitol* 2003; **97**: 23–9.

20 Apt W, Arribada A, Zulantay I *et al*. Itraconazole or allopurinol in the treatment of chronic American trypanosomiasis: the results of clinical and parasitological examinations 11 years post-treatment. *Ann Trop Med Parasitol* 2005; **99**: 733–41.

21 Zulantay I, Apt W, Valencia C *et al*. Detection of *Trypanosoma cruzi* in untreated chronic chagasic patients is improved by using three parasitological methods simultaneously. *J Antimicrob Chemother* 2011; **66**: 2224–6.

22 Wincker P, Bosseno MF, Britto C *et al*. High correlation between Chagas' disease serology and PCR-based detection of *Trypanosoma cruzi* kinetoplast DNA in Bolivian children living in an endemic area. *FEMS Microbiol Lett* 1994; **124**: 419–23.

23 Bravo N, Muñoz C, Nazal N *et al*. Real-Time PCR in faecal samples of *Triatoma infestans* obtained by xenodiagnosis: proposal for an exogenous internal control. *Parasit Vectors* 2012; **5**: e59.

24 Duffy T, Bisio M, Altcheh J *et al*. Accurate real-time PCR strategy for monitoring bloodstream parasitic loads in Chagas disease patients. *PLoS Negl Trop Dis* 2009; **3**: e419.

25 WHO. Chagas disease interrupted in Chile. *TDR, News* 2000; **61**: 10.

26 Bustamante J, Bixby L, Tarleton R. Drug-induced cure drives conversion to a stable and protective CD8+ T central memory response in chronic Chagas disease. *Nat Med* 2008; **14**: 542–50.

27 Coronado X, Zulantay I, Rozas M *et al*. Dissimilar distribution of *Trypanosoma cruzi* clones in humans after chemotherapy with allopurinol and itraconazole. *J Antimicrob Chemother* 2006; **58**: 216–9.

28 Toledo M, Bahia M, Carneiro C *et al*. Chemotherapy with benznidazole and itraconazole for mice infected with different *Trypanosoma cruzi* clonal genotypes. *Antimicrob Agents Chemother* 2003; **47**: 223–30.

29 Tuccori M, Bresci F, Guidi B *et al*. Fatal hepatitis after long term pulse itraconazole treatment for onychomycosis. *Ann Pharmacother* 2008; **4b2**: 1112–7.

30 Viotti E, Vigliano C, Armenti H *et al*. Treatment of chronic Chagas disease with benznidazole: clinical and serological evolution of patients with long-term follow-up. *Am Heart J* 1994; **127**: 151–62.

31 Apt W. Treatment of Chagas disease. In: Telleria J, Tibayrenc Med. *American Trypanosomiasis Chagas Disease. One Hundred Years of Research*. Amsterdam: Elsevier Science, 2011; 809–25.