

Case Report: Successful Treatment with Posaconazole of a Patient with Chronic Chagas Disease and Systemic Lupus Erythematosus

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Abstract. American Trypanosomiasis or Chagas disease (CD) is a neglected disease that affects Latin American people worldwide. Two old antiparasitic drugs, benznidazole and nifurtimox, are currently used for specific CD treatment with limited efficacy in chronic infections and frequent side effects. New drugs are needed for patients with chronic CD as well as for immunosuppressed patients, for whom the risk of reactivation is life-threatening. We describe a case of chronic CD and systemic lupus erythematosus (SLE) that required immunosuppression to control the autoimmune process. It was found that benznidazole induced a reduction, but not an elimination, of circulating *Trypanosoma cruzi* levels, whereas subsequent treatment with posaconazole led to a successful resolution of the infection, despite the maintenance of immunosuppressive therapy.

INTRODUCTION

One hundred years after its scientific description, American Trypanosomiasis or Chagas disease (CD) remains the largest parasitic burden in Latin America. The epidemiology of CD has changed because of the migratory trends at the beginning of the 21st century, and CD is emerging as a global infectious disease.^{1,2} Despite successful control measures in some regions of Latin America based on the prevention of the vectorial and transfusional transmission of the etiological agent (the protozoan parasite *Trypanosoma cruzi*),³ many challenges remain. First, there are limitations of currently available specific treatment, benznidazole (LAFEPE, Pernambuco, Brasil; ex-Roche) and nifurtimox (Bayer, Leverkusen, Alemania), because of limited efficacy in the chronic stage of the disease, which is the most prevalent clinical presentation of this condition,⁴ and common unwanted side effects. Second, there is a lack of biological markers for the early evaluation of antiparasitic drug efficacy and clinical response.

The latter aspect is particularly pressing for the management of adult patients with chronic disease because of three aspects. First, evaluation of the clinical response to specific treatment would require years or decades of follow-up. Second, conventional serology responds slowly to parasite elimination, and the lag time increases with the duration of the original infection.^{5–8} Third, in chronic infections, the levels of circulating parasites are often at or below the limit of the most sensitive direct parasitological methods, such as PCR.^{9–13}

Moreover, many patients with chronic CD have other health conditions, including immunosuppression, which can severely complicate the prognosis. Concomitant infections of *T. cruzi* and human immunodeficiency virus (HIV) are serious and have been well-described. Also, advances in other medical fields have led to increased use of immunosuppressive treatments for some conditions (malignancies and autoimmune diseases), and this generally increases the severity of CD clinical presentation.

Reactivation of CD in patients with acquired immunodeficiency syndrome (AIDS) is well-documented, and the need for treatment and secondary prophylaxis has led to several protocols for management for these dual infections, although currently there is no general consensus.¹⁴ There is also evidence that CD can be reactivated in patients with hemoproliferative malignancies,¹⁵ and as reported in one case, comorbidity with systemic lupus erythematosus (SLE) can occur.¹⁶ Although, to the best of our knowledge, acute reactivation of CD has not been documented in autoimmune disorders, the need for continuous immunosuppressive treatment generates a risk for reactivation or the potential development of severe chronic forms of disease in these patients.¹⁷

In this report, we describe benznidazole treatment failure in a patient with CD and SLE as well as subsequent successful treatment with posaconazole.

CASE DESCRIPTION

A 44-year-old Argentinean female with a 1-year history of arthritis and malaise was admitted to the hospital in April 2007 with fever, asthenia, and edema. The patient had previously suffered untreated high blood pressure and had been diagnosed with *T. cruzi* infection 20 years before admission.

Epidemiologically, the patient came from the Misiones province of northern Argentina, an endemic region for CD, and arrived in Spain 5 years before admittance. She never lived in a mud house, which is a risk factor for CD, but 25 years before admittance, she received a blood transfusion during her first delivery. In this case, the infection might have been caused by vector-borne transmission and/or by blood infected by *T. cruzi* that she received 25 years ago in a transfusion. Until April 2006, she remained asymptomatic, had not been followed-up for *T. cruzi* infection, and had never received specific treatment. The patient was admitted in our hospital from April 26 to May 5, 2007. At admission, abnormal laboratory values included leucopenia ($3.5 \times 10^9/L$), low hemoglobin (83 g/L), and increased erythrocyte sedimentation rate (90 mm/hour), whereas C-reactive protein was normal (1 mg/dL). In addition, elevated urea (110 mg/dL), and creatinine (3.2 mg/dL) were detected. Urine analysis showed cloudy urine with proteinuria (2,165 mg/24 hours), a white cell count of 15–20 per

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high-power field (HPF), granular casts (1–2/HPF), and many erythrocytes. Autoantibody screening showed high titers of antinuclear antibodies (1:640) with a homogeneous pattern and antibodies against double-stranded DNA (dsDNA) up to 200 U/mL (normal range = 0.0–19.9). There were also low complement titers of C4 (<0.07 g/L; normal range = 0.11–0.45), C3 (0.262 g/L; normal range = 0.820–1.870), and CH50 (2 U/mL; normal range = 34–71). Antiphospholipid antibodies, including lupus anticoagulant and anticardiolipin antibodies, were negative.

Kidney biopsy showed diffuse lupus nephritis class IV-G (A) with an activity index of 10/24 and a chronicity index of 5/12. A diagnosis of SLE with active lupus nephritis was made, and treatment with three pulses of methylprednisolone (1 g/day for 3 days followed by prednisone at 1 mg/kg/day slowly tapered) was started on April 27, 2007. It was followed by monthly intravenous cyclophosphamide (IVC; 750 mg/m² of body surface) for 6 months (from May to October 2007).

A control electrocardiogram (EKG) was normal, but echocardiography showed a concentric hypertrophy of the left ventricle, dilatation of the right atrium, mild tricuspid insufficiency, and mild pulmonary hypertension, probably caused by chronic hypertension.

Also at admission (April 2007), the patient was confirmed to be positive for *T. cruzi* infection using two different serological enzyme-linked immunosorbent assay (ELISA) tests: ELISAr with a ratio of 8.3 (BioELISA Chagas, Biokit S.A., Lliçà d'Amunt, Barcelona, Spain with recombinant antigens) and ELISAc with a ratio of 153 (an in-house ELISA with whole *T. cruzi* epimastigotes antigen)¹⁸ (Table 1). ELISA by Biokit was carried out following the instructions of the manufacturer. A cut-off was calculated by adding 0.300 to the mean absorbance of the negative control. The results are recorded as follows: positive, absorbance ratio sample/cut-off ≥ 1 ; negative, absorbance ratio sample/cut-off < 0.9; equivocal, absorbance ratio sample/cut-off ≥ 0.9 but < 1.0. For the ELISAc, the reaction was quantified as units (U) related to a positive serum, included in the plates, used as calibrator and arbitrarily set at 100 U. The cut-off was established at 20 U.

In this context, after the initial immunosuppressive treatment, weekly clinical and parasitological (polymerase chain reaction [PCR] and thick blood film) follow-ups were made. In May 2007, a real-time PCR (RT-PCR)¹⁹ for *T. cruzi* was positive (Table 1), which led to treatment with benznidazole of 5 mg/kg/day for 60 days starting simultaneously with the IVC treatment. The patient showed no clinical symptoms related to *T. cruzi*, and no significant side effects related to benznidazole were detected.

After six monthly pulses of IVC, the patient's symptoms improved, and there was complete response of lupus nephritis (normalization of the plasma creatinine levels and disappearance of hematuria and proteinuria). Six months after the end of benznidazole treatment (February 2008) and before the next round of monthly IVC immunosuppressive treatment, a RT-PCR test for *T. cruzi* was carried out and was again found to be positive, although the calculated circulating parasite levels were 10–100 times lower than in the pre-benznidazole assay (Table 1). Because of the required maintenance of immunosuppressive treatment with azathioprine (100 mg/day orally), the risk of development of neurological or cardiac complications, and the failure of the benznidazole treatment, an off-label treatment with posaconazole (Noxafil oral suspension; Schering Plough Corporation, Kenilworth, NJ) was suggested on a compassionate basis with the agreement of the Spanish Ministry of Health. After informed consent by the patient, a 400 mg per 12 hour for 90 days posaconazole treatment was given, starting 45 days before the start of the new IVC treatment. Posaconazole was well-tolerated, and *T. cruzi* blood PCR was consistently negative throughout the follow-up interval that was 13 months after the start of posaconazole treatment (nine consecutive negative PCR tests); immunosuppressive treatment was maintained during this time (Table 1). After two additional quarterly pulses of IVC and then a switch to azathioprine (100 mg/day), the patient presented normal renal function and no SLE recurrence in January 2010. It should be noted that, despite consistently negative blood PCR tests after posaconazole treatment, the levels of anti-*T. cruzi* circulating antibodies detected by conventional serology remained essentially unchanged (Table 1).

Other drug therapies concomitant with posaconazole treatment included hydroxychloroquine (200 mg/day), enalapril (20 mg/day), atenolol (100 mg/day), omeprazole (20 mg/day), and atorvastatin (40 mg/day). Currently, 2 years after initial diagnosis, the patient continues with prednisone (2.5 mg/day), azathioprine (100 mg/day), hydroxychloroquine (200 mg/day), and calcium plus vitamin D.

DISCUSSION

Benznidazole, a 2-nitroimidazole derivative, and nifurtimox, a 5-nitrofurant, are the only drugs currently available for specific CD treatment. In newborns, the drugs are well-tolerated and have an efficacy of $\geq 95\%$,²⁰ in acute adult infections, the efficacy is also high, but side effects increase with age. However, efficacy declines markedly with the duration of infection.²¹ Although several studies have shown that

TABLE 1
Serological and parasitological results for *T. cruzi* infection after treatment with benznidazole and posaconazole

| Date | Before BZD | After BZD | During treatment with posaconazole* | | | | | After treatment with posaconazole | | | |
|---------|------------|-----------|-------------------------------------|---------|---------|---------|--------|-----------------------------------|--------|----------|--------|
| | 5/17/07 | 2/6/08 | 3/18/08 | 4/28/08 | 5/16/08 | 5/23/08 | 6/6/08 | 6/18/08 | 8/5/08 | 10/13/08 | 4/6/09 |
| ELISAr† | 8.3 | 6.7 | 6.6 | 6.9 | 4.4 | 7.7 | 7.3 | 6.7 | 7.1 | 7.7 | 6.2 |
| ELISAc‡ | 153 | 99 | 109 | 114 | 100 | 114 | 102 | 104 | 98 | 118 | 114 |
| RT-PCR§ | Ct25 | Ct35 | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |

BZD = benznidazole; ELISA = enzyme-linked immunosorbent assay; r = recombinant, c = in house; RT-PCR = real-time polymerase chain reaction; Neg = negative values.

* Posaconazole treatment started on 3/17/08.

† The reaction results were quantified as a ratio following the manufacturer's instructions. Positive values ratio ≥ 1 .

‡ The reaction results were quantified as units (U) related to a positive serum used as the calibrator and was arbitrarily set at 100 U. The cut-off was established at 20 U.

§ RT-PCR reactions were performed in triplicate for each sample. Ct = cycle threshold (see Piron and others¹⁹). Positive values: Ct25 = 10 parasites/mL; Ct35 = 0.1–1.0 parasites/mL. Negative values: Ct = 45 (< 0.01 parasites/mL).

benznidazole is unable to induce parasitological cures in the majority of chronically infected adult patients,^{22,23} there is an increasing trend to offer antitrypanosomal treatment to infected adults with asymptomatic disease or early cardiomyopathy, because it has been shown in some studies that this drug can slow down the progression of cardiac lesions.²⁴ This could be explained by a reduction of the patients' parasite load and the associated inflammatory response at the sites of infection. The suboptimal response rates attained with benznidazole and nifurtimox in patients with established chronic infections are partially explained by the natural resistance of some *T. cruzi* strains²⁵ and by the drugs' inadequate pharmacokinetic properties.⁴ However, the role of the host immunological response on the drugs' activity is not yet fully understood.

Currently, specific treatment is universally indicated for reactivation in immunosuppressed patients with *T. cruzi* infection, and it might be indicated for preventing reactivation in the asymptomatic or indeterminate phase, although no consensus exists for the latter application. Severe fatal cases of meningoencephalitis and/or myocarditis have been described as caused by reactivation of *T. cruzi* infection in immunosuppressed patients, although both complications are almost never seen in immunocompetent patients with CD.²⁶ These unusual manifestations have also been reported in CD patients with immunosuppression because of other causes, such as immunosuppressive treatment post-transplantation.^{27,28} Different strategies for control of the reactivation of *T. cruzi* infection in immunosuppressed patients have been proposed. Benznidazole is the treatment of choice for reactivated CD in HIV-infected patients,²⁵ whereas for nifurtimox, there is much more limited experience. In patients with AIDS, secondary prophylaxis (benznidazole three times per week) is recommended, but it is often discontinued when CD4+ lymphocytes are above 200 cells/ μ L.¹⁴ Retreatment is recommended for HIV-infected patients who fail to respond or who relapse after initial antiparasitic therapy.²⁹

Currently, there is no consensus on the management of chronic CD patients that require immunosuppression by corticosteroids, despite the fact that such treatments are a critical reactivation risk. Some authors advocate the concomitant administration of anti-*T. cruzi* treatment, even in absence of acute CD symptoms (a strategy called primary prophylaxis),²⁷ whereas others are more conservative in the absence of clinical trials. Moreover, there is very little experience in patients with CD and concomitant autoimmune diseases. Knowing the unpredictable and usually long-term evolution of CD manifestations, treatment of immunosuppressed patients who exhibit detectable parasitemia seems to be a prudent therapeutic conduct. Currently available high-sensitivity methodologies, such as serial RT-PCR, allow for the early detection of rising circulating *T. cruzi* levels before the appearance of clinical symptoms of reactivation, which can lead to precocious specific treatment to avoid possible subsequent complications.³⁰⁻³²

New drugs approved for use in humans for other indications, such as new antifungal triazole derivatives that are selective inhibitors of *T. cruzi* ergosterol synthesis (acting at the level of C14 α sterol demethylase, CYP51), are promising alternatives for specific chronic CD treatment; these compounds have high anti-*T. cruzi* efficacy in vitro and in experimental animal models of both acute and chronic CD, and they are active against nifurtimox- and benznidazole-resistant strains of *T. cruzi*, even in immunosuppressed hosts.³³ The remarkable in vivo anti-

T. cruzi activities of these compounds are thought to result from their potent intrinsic activity against the parasite (minimal inhibitory concentrations against intracellular amastigotes in vitro in the low nanomolar to subnanomolar range) and special pharmacokinetic properties, such as long terminal half-life and large volumes of distribution.³⁴ Among the new triazole derivatives, posaconazole—currently registered in the United States, the European Union, and Australia for the treatment and prophylaxis of invasive fungal infections—is the most advanced candidate for a new anti-*T. cruzi* drug.

In our patient, an IVC immunosuppressive treatment was indicated for SLE and had satisfactory results, but specific treatment of the *T. cruzi* infection with benznidazole was unable to clear the circulating parasites, as indicated by a positive RT-PCR 6 months after antiparasitic treatment. For the reasons given above, posaconazole seemed an appropriate drug for treating the established *T. cruzi* infection of the patient, despite the fact that it is not currently registered as an antiparasitic drug. In immunocompetent patients, even serial negative *T. cruzi* blood PCR tests cannot prove the absence of intracellular parasites in the target tissues, but in the present case, the fact that parasitemia levels consistently stayed below the detection limit of our RT-PCR assay during and after posaconazole treatment, despite the maintenance of immunosuppressive therapy, strongly suggests a drug-induced parasitological cure of the patient. This interpretation is supported by the results of a recent study in a murine model of chronic CD where parasitological cures induced by benznidazole treatment were ascertained by the lack of parasitemia reactivation induced by cyclophosphamide, which strictly correlated with negative *T. cruzi* PCR tests in target tissues.³⁵ Also in accordance with this conclusion, the patient has not shown any sign of clinical CD symptoms during the follow-up. However, the levels of anti-*T. cruzi* antibodies detected by conventional serology (ELISA) were not significantly modified by posaconazole treatment (Table 1). Such "dissociation" between parasitological and conventional serological tests has also been found in previous studies of the response of chronic *T. cruzi* infections to specific drug treatments.³⁶ Our results showed that posaconazole had superior efficacy when compared with benznidazole in controlling a chronic *T. cruzi* infection compounded by sustained immunosuppression. Finally, posaconazole treatment was well-tolerated with no adverse events detected during or after 90 days of treatment.

Although posaconazole seems to be the best pharmacological alternative to benznidazole and nifurtimox for specific CD treatment, its current cost is too high for its widespread use in endemic countries; however, this limitation could potentially be negotiated, and other drugs with the same mechanism of action and similar pharmacokinetic profile but lower potential cost of goods could also enter clinical development for this condition in the future (see <http://www.dndi.org/press-releases/532-eisai-and-dndi-enter-into-a-collaboration.html>).

CONCLUSIONS

Patients with compatible epidemiological backgrounds and in need of immunosuppression must be tested for *T. cruzi* infection and clinical symptoms of CD. As suggested by others authors,⁹ PCR is now a standard tool for the diagnosis and follow-up of such patients. Although a negative PCR cannot prove eradication of the parasite, a positive PCR can provide

early unequivocal evidence of treatment failure and could help drive the consideration of other therapeutic options to prevent acute reactivations or chronic progression of CD. More effective and safer drugs for the specific treatment of CD, particularly in its chronic stage, are urgently needed. All infected patients, including those immunosuppressed, would benefit from such drugs. Posaconazole, already in the market for the treatment of invasive mycoses, has an excellent safety profile and superior anti-*T. cruzi* activity compared with benznidazole in experimental animal models of CD; therefore, it is a prime candidate for a safer and more effective treatment of this parasitosis. The results of the present study in an immunocompromised CD patient support this notion. A successful clinical development of this or similar compounds like anti-*T. cruzi* drugs would enormously improve the management of this disease, which affects millions of people and is becoming an emerging global disease.

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