Response to different benznidazole doses in animal models of chronic phase Chagas disease: a critical review

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Abstract

Chagas disease is a protozoan infection that was identified over a century ago. No drugs are available to treat the indeterminate and determinate chronic phases of the disease. Success of a drug design is dependent on correct biological evaluation. Concerning new drug designs for Chagas disease, it is essential to first identify the most effective, existing, experimental chronic protocols that can be used for comparison purposes. Here, we present a literature review regarding experimental models with chronic Chagas disease to evaluate the efficacy of benznidazole (BZN). We searched literature published in PubMed and Web of Science databases, using these keywords: animal model, BZN, Chagas disease, *T. cruzi*, and chronic phase, with no timeframe limitations. We excluded articles involving acute phase animal models and/or those without BZN treatment. The selected studies were conducted using different BZN concentrations (10mg-100mg) involving several different periods (5-70 days). Concentrations and durations of use are directly related to side effects, but do not prevent chronic tissue lesions. BZN use during the late/chronic phases of Chagas disease is unable to eliminate amastigote forms present in infected tissues. This study suggests the administration of a lower BZN concentration (<100mg/kg/day) during the chronic phase of the animal model, as this had been reported to result in fewer side effects.

Keywords: Benznidazole. Chagas disease. Chronic phase.

INTRODUCTION

Chagas disease is an endemic zoonosis that affects 8 million people worldwide, originating in Latin America, and endemic in 21 Latin American countries[1]. The reach of the parasite is expanding due to globalization and the increasing number of infections occurring in developed countries, such as the USA, Spain, and Japan. More than 100 years after it was first described, there remain only two available drugs for treatment, namely, benznidazole (BZN) and nifurtimox. These drugs have been in use for more than four decades, and the discovery of new drugs is urgently needed. However, in some countries, such as Brazil, BZN is the only available drug, which is highly active in the acute phase of the disease but its efficacy in the chronic phase of Chagas disease remains controversial. Moreover, there is little evidence to support BZN as totally effective in parasite elimination[2-4], and some studies have observed efficiency only in the indeterminate form of the chronic phase in children[5-7]. These drugs have also shown questionable efficacy in the chronic phase and several adverse reactions have been reported, mainly in adults, such as anorexia, weight loss, nausea, vomiting, insomnia, depression, convulsions, dizziness, headache, drowsiness, muscle pain, loss of balance, mental confusion, memory problems, peripheral neuropathies, mucosal edema, liver intolerance, and cutaneous manifestations[8-11]. Since discovering the disease, no drug has shown efficacy for the indeterminate and chronic phases of the disease[12-17]. One step towards achieving success in new drug development involves adequate animal *in vivo* testing. Development of a mouse model with chronic Chagas disease is difficult and depends on the choice of the animal, the strain of *Trypanosoma cruzi (T. cruzi)* the amount of inoculated parasite load, confirmation of the disease stage, time/dose of the drug in tests, and maintenance of animal survival at the end of the experiment. The current study presents the experimental animal models that have been used and highlights the effect of BZN in the indeterminate and determinate chronic infection phases.

The most recent *in vivo* model that can identify the parasite during the experimental procedure is based on modified

parasites that are highly sensitive to *in vivo* imaging, using a bioluminescence imaging system based on *T. cruzi*[18-20]. However, this methodology is not extensively used in every laboratory. **Table 1, Table 2** and **Table 3** indicate the studies that have reported the effect of BZN in indeterminate/chronic infections and the experiments for chronic infections in several *T. cruzi* strains (**Table 1**[17-19,22], **Table 2**[17,18,21-23], **Table 3**[18,24-29]).

Immunosuppression alters the natural development of the disease, predetermining the development or reactivation of opportunistic infections. This is especially true for Chagas disease[30,31], particularly in animal models, where immunosuppression is simply applied to evaluate a sterile cure. This effect occurs despite efforts to produce a perfect chronic phase model that involves the use of BZN.

A current strategy in use involves therapeutic doses of BZN as part of a combination therapy for Chagas disease. This entails therapeutic regimens with low concentrations to decrease toxicity and consequently reduce side effects due to typical BZN therapy. One study[32] has evaluated the effect of an antihypertensive medication in association with a standard anti-*T. cruzi* drug. That study used enalapril (25mg/kg) and BZN (100mg/kg) separately and together for 30 days in animal models infected with the VL-10 strain of *T. cruzi*, mainly to evaluate chronic cardiac inflammatory parameters, levels of chemokines (CCL2, CCL5), IL-10, creatine kinases (CKs), and C-reactive protein serum 120 days post-infection. The authors reported that the combination of enalapril and BZN could decrease the biomarker levels of CK, CK-MB, and C-reactive proteins during the chronic phase of the disease, and also reduce the intensity of inflammatory infiltrates in the heart. Two years later, the same authors[33] evaluated this same therapeutic combination (enalapril/BZN) during the chronic phase of the disease in an animal model infected with the VL-10 strain of *T. cruzi*. However, in their latter study they used several different concentrations: BZN (100, 80, and 60mg/kg), enalapril (25, 20, and 15mg/kg), and both (100 + 25; 80 + 20; 60 +15mg/kg, respectively). These concentrations were administered for 20 days orally by gavage, and euthanization occurred 120 days post-infection. This was performed to decrease toxicity and cardiac lesions, as well as to produce the same anti-*T. cruzi* effect. Their data demonstrated that a therapeutic combination resulted in an approximate 20% decrease in the parameter concerning inflammatory infiltrates when compared to standard BZN therapy. However, the therapeutic combination (100 +25 and 80 +20mg/kg) enhanced IL-10 levels, and also decreased cardiac inflammatory infiltrates evaluated in the histopathological analysis.

TABLE 1: Experimental studies involving chronic phase Chagas disease in animal models with immunosuppression: BZN = 100mg/kg/day.

T. cruzi strain	Mice	Inoculum	Start of treatment (dpi)	BZN (mg/kg/day)	Treatment (duration)	Immunosuppression	Results			Reference
							Parasitemia (reactivation)	PCR, or Serological test	Amastigotes presence	
JRcl4*	C3H	10^4	161**	100	5	▲	positive	ND	ND	17
CL Brener*	BALB/c	10^3	90**	100	20 days	β	negative	ND	ND	18
CL Brener*	BALB/c and CB17 SCID	10^3	103**	100	5 days	κ	positive	negative (PCR)	Negative	
CL Brener*	BALB/c and CB17 SCID	10^3	103**	100	10 days	κ	positive	negative (PCR)	Positive	19
CL Brener*	BALB/c and CB17 SCID	10^3	74**	100	20 days	λ	negative	negative (PCR)	Positive	
CL Brener or Y	C57BL/6	10^3	35***	100	10 days	θ	ND	positive (PCR)	ND	
CL Brener or Y	C57BL/6	10^3	35***	100	15 days	θ	ND	positive (PCR)	ND	22
CL Brener or Y	C57BL/6	10^3	35***	100	20 days	θ	ND	negative(PCR)	ND	
CL Brener or Y	C57BL/6	10^6	35***	100	20 days	θ	ND	negative (PCR)	ND	

BZN: benznidazole; ***T. cruzi:*** *Trypanosoma cruzi*; **dpi:** days post-infection; **PCR:** polymerase chain reaction; **ND**: not determined; ▲: cyclophosphamide (200mg/kg) on days 175, 179, and 183 dpi; **bid:** bis in die (twice daily); **β**: cyclophosphamide (200mg/kg) on days 124, 127, 130, and 133 dpi; **κ**: cyclophosphamide (200mg/kg) on days 124, 128, and 132 dpi; **λ**: cyclophosphamide (200mg/kg) on days 113, 118, and 128 dpi; **θ**: cyclophosphamide (200mg/kg) four cycles of cyclophosphamide with each cycle lasting one week. *Bioluminescent bloodstream-form of *T. cruzi*. **Chronic phase; ***Late acute phase.

TABLE 2: Experimental studies involving chronic phase Chagas disease in animal models with immunosuppression: BZN ≤ 50 mg/kg/day.

T. cruzi strain	Mice	Inoculum	Start of treatment (dpi)	BZN (mg/kg/day)	Treatment (duration)	immunosuppression	Results			Reference
							Parasitemia (reactivation)	PCR, or Serological test	Amastigotes presence	
CL Brener*	Balb/c	10^4	124 or 126**	10	10 days	•	positive	ND	ND	
CL Brener*	Balb/c	10^4	124 or 126**	10	20 days	•	positive	ND	ND	
CL Brener*	Balb/c	10^4	124 or 126**	30	5 days	#	positive	ND	ND	17
CL Brener*	Balb/c	10^4	124 or 126 ***	30	10 days	†	positive	ND	ND	
CL Brener*	Balb/c	10^4	124 or 126**	30	20 days	†	negative	ND	ND	
CL Brener*	Balb/c	10^4	114**	50 bid	10 days	α	negative	ND	ND	
CL Brener*	Balb/c	10^3	90**	10	20 days	β	positive	ND	ND	18
Y*	Balb/c	10^4	126**	50	28 days	ε	negative	ND	ND	21
CL Brener or Y	C57BL/6	10^3	35***	30	20 days	θ	ND	positive (PCR)	ND	
CL Brener or Y	C57BL/6	10^3	35***	10	20 days	θ	ND	positive (PCR)	ND	22
Y and Peruvian	Swiss	4×10^3	At 5 to 6 months**	50	5 days	γ	positive	positive (serological)	positive	
21SF	Swiss	4×10^3	At 5 to 6 months**	50	5 days	δ	positive	positive (serological)	Positive	23
Bolivia, Colombia and Montalvania	Swiss	5×10^4, 3×10^5	At 3 to 5 months**	50	5 days	γ	positive	positive (serological)	Positive	

BZN: benznidazole; _T. cruzi_: _Trypanosoma cruzi_; **dpi:** days post-infection; **PCR:** polymerase chain reaction; **ND**: not determined; •: cyclophosphamide (200mg kg−1) by i.p. injection every 4 days for a maximum of 3 doses; **#**: cyclophosphamide (200mg/kg) on days 138, 142, and 146 dpi; †: cyclophosphamide (200mg/kg) on days 154, 158, and 162 dpi. α: cyclophosphamide (200mg/kg) on days 135, 139, and 143 dpi; β: cyclophosphamide (200mg/kg) on days 124, 127, 130, and 133 dpi; ε: cyclophosphamide (200mg kg−1) by i.p. injection during 2 days at 3-day intervals; θ: cyclophosphamide (200mg/kg) four cycles of cyclophosphamide with each cycle lasting one week; γ: Azathioprine (2mg/kg/day) gavage, betamethasone (1mg/kg/day), cyclosporine (16, 10, 8 and 6 mg/kg/day); δ: Azathioprine (10mg/kg/day) gavage, betamethasone (2 mg/kg/day), cyclosporine (30mg/kg/day) gavage. *Bioluminescent bloodstream-form of _T. cruz_i. **Chronic phase. ***Late acute phase.

In addition to combination therapy, other strategies to overcome the extended duration and toxicity of treatment have included identification of new therapeutic targets for Chagas disease, drug discovery, drug repositioning, and re-dosing regimens for current drugs. In this context, researchers have optimized Chagas therapy using BZN/itraconazole in different doses (50, 75, and 100mg/kg), and evaluated the therapeutic effect in an animal model infected with the Y strain of _T. cruzi_. When compared to compound-only groups (100mg/kg), the combination therapy (BZN/itraconazole) showed a decrease in the treatment duration required to reduce the trypomastigote forms in animals. The concomitant treatment

TABLE 3: Experimental studies involving chronic phase Chagas disease in animal models without immunosuppression.

T. cruzi strain	Mice	Inoculum	Start of treatment (dpi)	BZN (mg/ kg/day)	Treatment (duration)	immunosuppression	Results			Reference
							Parasitemia (reactivation)	PCR, or Serological test	Amastigotes presence	
CL Brener*	BALB/c	10^3	66**	100	5 days	-	negative	ND	ND	18
Clone CL Brener B5	BALB/c	10^2	90**	20	20 days	-	ND	ND	positive	
Clone CL Brener B5	BALB/c	10^2	90**	50	20 days	-	ND	ND	positive	24
Tulahuen and SGO-Z12	Swiss	5 x 10	180**	100	30 days	-	negative	ND	ND	25
H510C8C3 clone	148 CF1	10^5	90**	25	60 days	-	negative	negative (PCR) / positive (serological)	negative	26
Colombian	BALB/c	10^2	45**	100	7 days followed weekly for 8 months	-	ND	ND	positive	27
20 Different Clonal Genotypes	BALB/c	10^4	90**	100	20 days	-	positive	positive (serological)	ND	28
Seven clones – Colombian resistant Strain	Swiss	5 x 10^4, 1 x 10^5	20***	100	70 days	-	positive	positive (PCR and serological)	ND	29

BZN: benznidazole; *T. cruzi: Trypanosoma cruzi*; **dpi:** days post-infection; **PCR:** polymerase chain reaction; **ND**: not determined; **-:** without immunosuppression. *Bioluminescent bloodstream-form of *T. cruzi*. **Chronic phase. ***Late acute phase.

also showed excellent immunomodulatory activity, mainly at the concentration of 75mg/kg, with a four-fold enhancement reported compared to the compound-only groups (100mg/kg)[34].

Researchers[35] have evaluated the concomitant treatment of BZN/pentoxifylline (25/20mg/kg, respectively) during 30 consecutive days with mice chronically infected with the Colombian strain of *T. cruzi*. The combination therapy showed a reduction of approximately 79% of amastigote nests, which is 3% more active than standard therapy with BZN (76%). A decrease in myocarditis, fibrosis, TNF expression (37%) in heart tissue, and improved electrical changes were also shown. These studies reinforce the importance of investigating previously-marketed drugs, particularly concomitant therapy with BZN against *T. cruzi*. This approach may provide a better quality of life for patients with Chagas disease and, for example, improve cardiac inflammatory levels, reduce biomarkers, and decrease treatment duration, as well as its concentration. As such, further studies concerning concomitant therapy for chronic Chagas disease are essential.

As well as smaller mammals such as rabbits and rats[36,37], larger animal models including dogs and non-human primates[38-50] are also used for experimental studies, mainly in Chagas disease, due to these mammals' capacity to host parasites. Dogs and non-human primates (baboons, macaques, and capuchin monkeys) have been important experimental models to study the pathogenesis of Chagas disease, mainly in regard to the immunopathogenic mechanisms involved in the chronic phase of *T. cruzi* infection[38,42-50]. Electrocardiography alterations[39,40,47,49] have been reported in studies of dogs and non-human primates infected with several *T. cruzi* strains as well in studies using BZN chemotherapy in dogs who received the same regimen as humans (7mg/kg), corroborating with the outcomes of other clinical trials[42]. Despite the variety of available experimental assays intended to simulate the *T. cruzi* infection, mice models have demonstrated more advantages than other experimental models, due to ease of handling, housing, greater sample numbers, and low cost, as well as the need to use a smaller drug quantity for the biological assays. In contrast, with larger animal models, the number of animals in a study is associated with ethical and cost considerations.

Considering that complete elimination is unlikely, the concentration of BZN should be decreased in the chronic phase of this disease in accordance with all the observed examples.

Treatment can then be completed without interruption while enabling parasite reduction, and with less damage to tissue.

LIMITED EFFECT IN HUMANS

Human treatment with BZN is limited due to side effects, and BZN does not provide a cure for the chronic phase of Chagas disease. BZN administration using several different doses and treatment durations has shown no cure for patients in the chronic phase. In a clinical trial conducted with 195 patients using different BZN doses of between 50 and 500mg/day (equivalent of 5mg/kg/day), approximately 30% of patients who received between 50 and 200mg/day discontinued treatment because of treatment side effects[51].

Several studies conducted in chronically-infected patients using different concentrations of BZN showed adverse effects, and treatment discontinuity occurred[52-55]. One study involved 80 asymptomatic patients with Chagas disease who were treated with BZN (5mg/kg twice daily for 60 days)[56]. The efficacy of BZN was evaluated over a period of three years, and only 5% of patients presented improvements. Another study[57] monitored 13 patients chronically infected with Chagas disease. They were treated with BZN 5mg/kg for 60 days. The accompaniment this patients lasted for more than four years and showed a decrease in the number of anti-*T cruzi* antibodies in 69% of patients (9 of 13 patients).

Researchers at the Chagas Disease Center in Buenos Aires[58], Argentina, evaluated 566 patients (range, between 30 and 50 years of age), with 3 positive results on serologic tests (no cardiac failure). Half (n=283) of the patients received 5mg/kg of BZN over 30 days, and the other half (n=283) went without treatment. The study reported that BZN treatment increased negative seroconversion in the chagasic patients and decreased the disease progression, principally reducing cardiac alterations due to the parasite. This result confirmed the limitations of BZN therapy in the chronic phase of Chagas disease, but should not be used as an argument for inefficacy.

THE TRANSITION TO CLINICAL TRIALS: A CHALLENGE FOR THE EXPERIMENTAL MODELS

In recent years, results from clinical trials involving Chagas disease have been reported and new azole derivatives such as posaconazole, ravuconazole, and E1224 (ravuconazole prodrug) have been evaluated. Using crystallographic studies (PDB code: 3K1O), posaconazole demonstrated good enzyme inhibition and good affinity. The drug showed activity against *T. cruzi* during *in vitro* studies against the epimastigote and amastigote forms of the parasite, and also during *in vivo* studies in the chronic model of the disease. Furthermore, the compound showed activity against drug-resistant strains of *T. cruzi*, such as the Y and Colombian strains. Posaconazole has also been evaluated in clinical phase studies (ClinicalTrials. gov, NCT01377480) in Argentina, Bolivia, and Spain, and has been compared to BZN in adults with chronic Chagas disease. Preliminary results of these studies have shown that posaconazole was clearly inferior compared to standard therapy, possibly due to lower systemic bioavailability. After reporting this result, the pharmaceutical company, Merck, began a second attempt to investigate the co-administration of posaconazole and BZN[59-65]. However, researchers have recently reported that the drug candidate posaconazole was less active than BZN in highly sensitive models of bioluminescent *T. cruzi*[66]. In the same way, ravuconazole (8) and compound E-1224 (9) (a ravuconazole prodrug) have also shown potent *in vitro* activity against *T. cruzi*. Although ravuconazole has an unfavorable pharmacokinetic profile in animal models (characterized with a very short elimination half-life), its pharmacokinetic parameters in humans have led to clinical trials of E-1224 (ClinicalTrials. gov NCT01489228). The results of these trials demonstrated that this compound did not remain effective after one year of treatment when compared to BZN. In addition, the incidence and severity of adverse effects at high doses led to discontinuation of treatment in the phase II clinical trial[67-70].

Despite efforts, and the number and quality of available experimental models intended to simulate Chagas disease, the results were disappointing in the clinical trials. Specifically, more confident predictions of the efficacy of these new drugs is required prior to starting clinical trials. Therefore, it is extremely important that better and safer methods are identified to improve the transition to clinical trials.

SCIENTIFIC CHALLENGES

More active compounds to treat the chronic phase of Chagas disease are urgently required and toxicity needs to be a strongly considered factor in the development of these compounds. The use of drug combinations that eliminate the intracellular parasites should not cause large quantities of tissue lesions. The best experimental protocol is one that effectively relates to human response.

The data shown here suggest that, for an experimental protocol in animals chronically infected with Chagas disease, treatment at lower doses (<100mg/kg/day) of BZN is more advantageous and may be used as a basis to compare new drugs with BZN during the chronic phase of Chagas disease.

Conflict of interest

The authors declare that there is no conflict of interest.

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