Chapter 8

Efficacy and Safety of Chagas Disease Drug Therapy and Treatment Perspectives

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Abstract

Chagas disease, also known as American trypanosomiasis, is a neglected disease caused by the protozoan parasite *Trypanosoma cruzi*. The disease affects about 6–7 million people worldwide, mostly in Latin America. Although Chagas disease was discovered more than 100 years ago, and the first treatments over 40, only 2 drugs were used to treat this pathology, it is still considered one of the neglected diseases. In this chapter, the subjects related to conventional etiological therapies, benznidazole and nifurtimox, such as the drug, the mechanism of action, the therapy schedule for treatment, efficacy and safety and their adverse effects will be discussed. Additionally, it will address alternative therapies of comorbidities related to the progression of Chagas' disease in patients with chronic disease, such as heart disease and dysfunction of the digestive system. Finally, novel pharmacological strategies and their related compounds will be reviewed accounting for their progression in pharmacological studies and their success rate.

Keywords: Chagas disease, benznidazole, nifurtimox, symptomatic treatment, new strategies

1. Introduction

Chagas disease or American trypanosomiasis is caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). Endemic largely in Latin American countries, it is transmitted primarily by vectors, the insect vector triatomine, also known as "kissing bug". It is estimated that 6–7 million people are infected worldwide, and that more than 10,000 people die each year as a result of the disease, with the highest number of cases in Latin American countries [1].

Although Chagas disease has been discovered more than 100 years ago, there are currently only two drugs used to control Chagas disease (CD), benznidazole and nifurtimox [2–4]. Benznidazole has a similar efficacy profile to nifurtimox. They have high cure rates in the acute phase and at the beginning of the chronic phase, being little or ineffective in the late chronic phase [5]. Benznidazole and nifurtimox belong to the nitro heterocycles class of drugs; however, each presents different mechanisms of action that are considered very aggressive and may cause several adverse effects.

In the chronic phase of CD, the treatment at this stage aims to reduce parasitemia and prevent complications that may lead to progression of visceral lesions. However, the administration of benznidazole and nifurtimox remains controversial [5, 6]. To treat the cardiac and gastrointestinal manifestations attributed to this phase of the disease, it requires specific pharmacological approaches, highlighting the combination of diuretics, angiotensin-converting-enzyme inhibitors or angiotensin receptor blockers, and adrenergic beta blockers. The choice of palliative treatment is in relation to the symptoms of the patient.

Currently, the main treatment for CD is benznidazole and nifurtimox and both compounds are effective in the acute phase to reduce parasitemia and the persistence and the clinical severity of the disease [5]. However, in most countries, benznidazole is the primary choice to begin the treatment, since it has shown less adverse events. The predominant reasons to use nifurtimox are the occurrence of benznidazole resistance or its unavailability [6, 7]. Benznidazole can achieve over 70% of cure in cases of congenital and acute phase, while nifurtimox reaches 80% efficacy. However, these compounds have limited efficacy in the chronic phase, with efficacy rates of only 6–10% [8–10]. Treatment complications include, need for increasing doses, duration varying according to the stage of infection, and high occurrence of adverse effects caused by the long-term treatment with high doses. In addition, resistance may occur depending on the *Trypanosoma. cruzi* (*T. cruzi*) strain [10].

Although the aforementioned therapy is over 40 years old, there are limitations in this first line of treatment for CD. Several studies aimed to bring new options to its arsenal, mainly for the chronic phase. Among them, are drugs used to treat other conditions, such as posaconazole, and new compounds, such as E1224. Each of these has been tested in clinical trials. Other strategies have been suggested as groundbreaking approaches to eradicate, or at least control, CD. These include drugs, such as cruzipain and trypanothione reductase which specifically targets the ergosterol biosynthesis pathway.

Considering this the classical treatment of CD, with a focus on the evaluation of efficacy and safety of the current drug therapy, will be properly addressed in this chapter. In addition, a review of new pharmacological therapies will be discussed.

2. Etiological treatment: benznidazole

2.1. Treatment history

Until the publication of the *Manual de Doenças Tropicaes e Infectuosas* in 1935 by Carlos Chagas and Evandro Chagas (Manual of Tropical and Infectious Diseases), there was no

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pharmacological treatment available for trypanosomiasis. Drugs with trypanocidal activity have been investigated by a number of researchers; however, without success [11]. In 1936, quinolinic compounds were successfully used to treat an acute case of trypanosomiasis [12]. In the following years, nitrofurazone was administered to treat trypanosomiasis in mice and achieve efficacy rates between 20 and 100%, depending on the therapeutic schedule [13]. These results motivated the experimental trials in humans, in which nitrofurazone demonstrated to be effective against trypanosomes in the circulating blood, cerebrospinal fluids [14] as well as other promising clinical results [15, 16].

Novel studies were performed to identify an alternative therapeutic schedule and main side effects [17, 18]. In 1962, Rio de Janeiro was the host city for the "Meeting of Debates on Chagas Disease", where the standardization of the methodology and establishment of criteria for the evaluation of attempted pharmacological treatments was discussed. Nifurtimox and benznidazole remain as the only two drugs available for treatment of CD [19].

2.2. The drug

Benznidazole (BNZ) N-benzyl-2-(2-nitro-imidazol-1-yl)acetamide (**Figure 1**) is a nitro heterocyclic drug. It was nitroimidazolic derivative synthesized in the early 1970s by Wineholt and Liebman and produced by Hoffman-La Roche, Switzerland. In 2003, the rights and manufacturing technology of BNZ was granted to the Laboratório Farmacêutico do Estado de Pernambuco (*LAFEPE*) – Recife/PE – Brazil [5, 9, 10, 20, 21]. According to the Biopharmaceutical Classification System, BNZ is categorized as class 4, thus exhibiting limited and/or variable absorption due to the characteristics such as low solubility and low permeability. Therefore, there are interests in strategies to improve the absorption rate, hence increasing its bioavailability [22, 23].

BNZ is administered orally in a tablet form of 100 mg for adults and 12.5 mg tablets for children [24]. The drug is fully absorbed in the gastrointestinal tract and the plasma peak concentration was achieved within 2–4 hours. The half-life is 12 hours and its metabolites were eliminated in urine and feces [5, 10, 20].

2.3. Mechanism of action

The mechanism of action of BNZ is not fully understood [9, 25]. However, some reports associate its action with the formation of free radicals and/or electrophilic metabolites. In the nitroimidazole derivatives, the reduction of the nitro group (NO_2) in the amino group (NH_2)

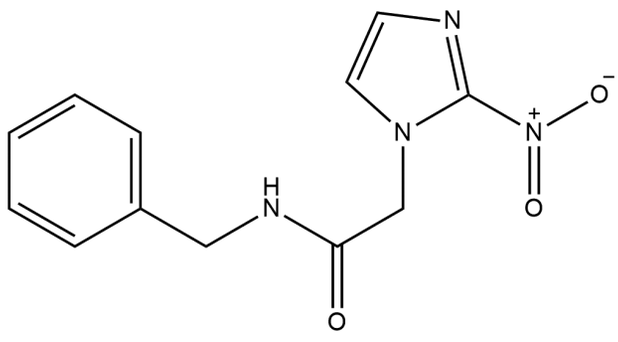

Figure 1. Chemical structure of benznidazole.

occurs by the action of nitroreductase present in protozoan cells or bacteria. This reaction leads to the formation of radical intermediates, as well as electrophilic metabolites. The process is initiated by the action of the enzyme NADPH cytochrome P450 reductase, which acts on the nitro group (R-NO_2) of the molecule nitroimidazole, inducing the production of an intermediate nitro radical (R-NO_2) and the formation of hydroxylamine (R-NHOH). These intermediates act on the covalent modification of macromolecules, such as DNA, causing fragmentation in the chain and destabilization of the helix, inhibiting DNA synthesis leading to cell death of protozoa parasitic and/or bacterial. In addition to this alteration in DNA, it involves the modification of other macromolecules, such as lipids and proteins, affecting *T. cruzi* metabolism. An additional mechanism of action of BNZ is the increase in phagocytosis, leading to lysis and inhibition of *T. cruzi* growth through an interferon-gamma (IFN-γ)-dependent mechanism and through the enzyme NADH-fumarate reductase, respectively [9, 11, 26–28].

Concomitantly, the electrophilic metabolites mentioned above due to their high reactivity and low specificity, may present human host action and cytotoxic effects observed during the treatment of patients [27–29].

2.4. Therapeutic schedule

The dosage of BNZ in the acute phase of the disease should be provided in two or three administrations daily, during the 60-day period. The dose is established according to the patient's age and weight. For adults, it is recommended 5 mg/kg/day, whereas for children weighing less than 40 kg, 5–10 mg/kg/day is recommended. Furthermore for children, the therapeutic regimen should be as small as possible to achieve therapeutic adherence. A recommendation for infants is 10 mg/kg/day. In congenital infections, the treatment is indicated for children born to mothers who were serologically positive for CD with the presence of *T. cruzi* in umbilical cord blood, serum-specific IgM soon after birth, and IgG after 6 months. In the newborn, the recommended etiologic therapy is the administration of BNZ twice a day for 30 days, it is suggested to be provided at 5 mg/kg/day [5, 11, 20, 30–32].

In the chronic phase, the dosage is 5–7 mg/kg/day divided into two administrations with an interval of 12-hour between doses, and for 30–60 consecutive days [5, 20].

2.5. Efficacy and safety

It is considered as the first choice treatment because it exhibits a better safety and efficacy profile when compared to nifurtimox. Moreover, there is evidence of greater efficacy [7, 28]. It has antiprotozoal and antibacterial performance, acting against the trypomastigote and amastigote forms [5].

In the acute phase, if the treatment is initiated immediately following the confirmation of the presence of *T. cruzi* in the direct examination of the peripheral blood, the cure percentages are greater than 70–80%, independent of the transmission route [5, 7, 9, 33]. It should be administered to patients in the early chronic phase, children less than 12 years, laboratory accidents and congenital infection. In newborns treated during the first year of life, the chances of cure are greater than 90%; otherwise, in the late chronic phase the patient has lower chances of cure (~10%) [20, 34–36].

Two placebo-controlled clinical trials were performed in the USA in children between 6 and 12 years old for the evaluation of treatment using BNZ, in which the results were satisfactory. In the first trial, 60% of the children treated had a change from positive to a negative in serology for CD, compared to 14% of them receiving placebo. In the second trial, similar results for children treated with BNZ showed a 55% change from a positive to a negative antibody test, compared to 5% of those receiving placebo [37].

For treatment in cases of organ transplantation, it is essential to know if the donor or recipient presents positive serology and can transmit or reactivate the infection. In cases of absolute requirement for transplantation, the serum-reactive donor in which the recipient is serologically negative for a chagasic infection, the donor should receive BNZ therapy following the traditional dosing regimen, within 60 days prior to transplantation. For the recipient following transplantation, it is recommended to initiate therapy by performing serological tests over time. There is a serum conversion with the treatment for the acute phase. If an acute infection is detected, the treatment need to be started [38–40].

Immunocompromised patients, carriers of hematological malignancies, users of immunosuppressive drugs, or those co-infected with the acquired immunodeficiency virus may reactivate CD. The treatment is the conventional treatment, lasting 60 days, and depending on the clinical conditions of the patient, it may be increased to 90 days [40–42].

When accidental infection occurs, the individual who accidentally had contact with contaminated materials; needle puncture, contact in lesions, wounds or mucous membranes, or any other means that indicates the possibility of having been infected by the parasite, is evaluated by serological tests and treatment begins immediately. During the 10–15 days of the treatment period, the serological tests will be repeated [5, 11].

At the beginning of the chronic phase in children, treatment follows the same reasoning for acute phase cases in children younger than or equal to 12 years with positive serology. For adults, therapy is recommended to prevent or reduce the progression of CD in more severe forms, such as cardiomyopathy, and to prevent congenital transmission in pregnant women [5, 11, 20, 43]. Its use in the chronic phase has generated vast discussions. The BENEFIT project – Benznidazole Evaluation for Interrupting Trypanosomiasis, is an international multicenter, randomized, double-blinded, placebo-controlled trial of BNZ for the treatment of patients with mild to moderate Chagas cardiomyopathy. This study has conducted and produced results that indicated that the use of BNZ in the chronic phase significantly reduced the detection of circulating parasites; however, did not attenuate the cardiac clinical progression [20, 33].

Treatment for patients older than 50 years should take into account the risk of toxicity of the drug against the benefits of the therapy individually [6, 20].

2.6. Adverse effects

Therapy with BNZ contains some challenges, such as the large doses administered, time duration varying according to the stage of infection, and high occurrence of adverse effects. These challenges are less frequent when compared with nifurtimox (**Table 1**). The adverse effects in patients receiving BNZ may be classified into three groups: manifestations of

Adverse effects	Benznidazole	Nifurtimox
Anorexia and weight loss	5–40%	50–75%
Nausea	0–5%	15–50%
Vomiting	0–5%	15–26%
Peripheral neuropathy	0–30%	2–5%
Leukopenia	Rare: <1%	Rare: <1%

Table 1. Frequencies of adverse effects associated with benznidazole and nifurtimox.

hypersensitivity (dermatitis with rashes, generalized or peritoneal edema, fever, lymphadenopathy, muscle and joint pain), bone marrow depression (agranulocytosis, neutropenia, and thrombocytopenic purpura), and peripheral polyneuropathy (paresthesias and polyneuritis) [7, 11].

Adverse effects of dermatological cause appear in approximately 30% of the patients being treated with the drug. Rashes occur due to photosensitization, dermatitis is usually mild to moderate and may be treated with topical systemic corticosteroids. However, treatment should be discontinued immediately in cases of severe or exfoliative dermatitis or associated with fever and adenopathy. Bone marrow suppression is rare, but if occurs the treatment should be discontinued immediately. Additional adverse effects include weight loss, nausea and/or vomiting, anorexia, and insomnia [8, 11, 20, 29, 43].

Treatment is contraindicated in pregnancy and in patients with severe renal or hepatic impairment [11, 20, 44].

According to the World Health Organization (WHO), the ideal requirements for treatment are: parasitological cure in both phases (acute and chronic), effective doses in single or few doses, low cost, no side effects or teratogenic effects, without hospitalization, and induction of resistance. Until now, there is no drug for the treatment of CD that meets each of these WHO requirements.

3. Etiological treatment: nifurtimox

3.1. The drug

Nifurtimox (NFX) (**Figure 2**) belongs to the class of nitrofuran compounds. Her-Linger, Mayer, Petersen and Bock from Bayer™ synthesized it in 1962 in Germany. This was the first drug designed to treat trypanosomiasis, such as sleeping sickness and Chagas diseases [34]. Its production was interrupted in 1980s due to the reduction on world demand [11]. In 2009, the WHO Expert Committee on the Selection and Use of Essential Medicines recommends the inclusion of NFX in the model list of essential medicines (EML) and Bayer resumed the production of NFX [45]. Bayer, through WHO, still provides NFX worldwide in 120 mg and 30 mg tablets under the brand name Lampit [46], in United States and it is redistributed by Center for Disease Control and Prevention (CDC) [47].

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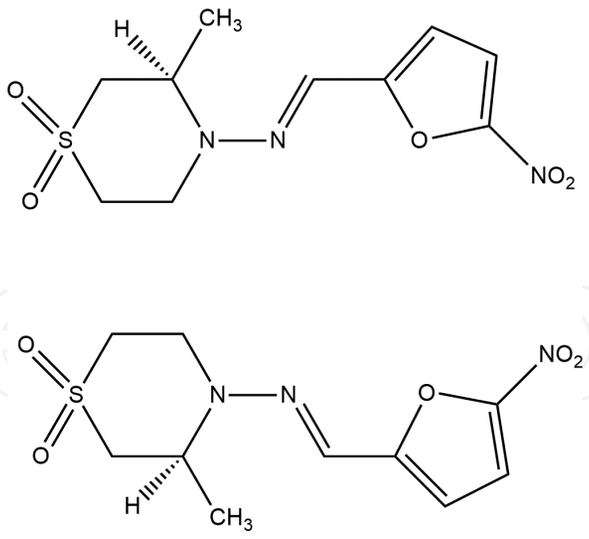

Figure 2. Chemical structure of nifurtimox.

NFX is a 5-nitrofuran (3-methyl-4-(5′-nitrofurfurylideneamine) tetrahydro-4H-1,4-tiazine-1,1-dioxide. NFX possesses an asymmetric center, but it is used as a racemic mixture since pure stereoisomers were not more active or less toxic.

NFX is well absorbed following oral administration. The plasma levels range from 10 to 20 mM and lower concentrations are found in urine and tissues. The therapeutic schedule must vary according to the patient's age and disease phase [9, 11, 48, 49].

3.2. Mechanism of action

The mechanism of action of NFX is based on cellular damage originating from the production of nitro anion radical through two pathways: through a redox cycle with the formation of $O\cdot^{-}$ (superoxide anion) and its reduction to the corresponding amine derivative [50–52].

This mechanism was thoroughly studied by Do Campo and colleagues [51, 53–58]. The reduction of a nitro group by nitroredutase (NTR) is fundamental for NFX mechanism of action. This enzyme catalyzes the reduction of 2-electrons of the compound, producing a nitrous intermediate, followed by a second 2-electron reduction to generate a hydroxylamine. This derivative can directly lead to cell damage, or generate other cytotoxic agents (**Figure 3**) [52, 59]. Since the *T. cruzi* detoxification mechanism is insufficient, it becomes more susceptive to reactive nitro compounds [60–62]. The presence of the nitro reductase type I in the parasite is the main responsibility for NFX selectivity. This enzyme is absent in mammals, reducing the formation of toxic agents in human.

3.3. Therapeutic schedule

Several therapeutic schedules were evaluated in the past years. The doses vary between 5 and 30 mg/kg/day, in extended therapies of 30–120 days [63–67]. Based on these experiments and considering the efficacy/tolerance ratio, the ideal therapeutic schedule of NFX is 8–10 mg/kg/day

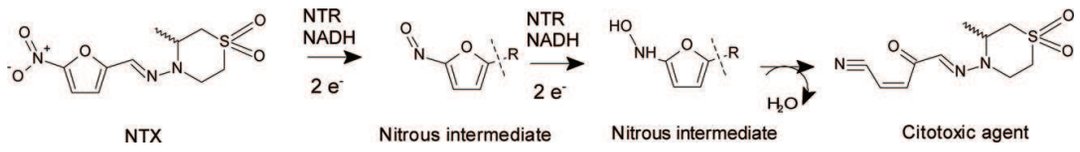

Figure 3. Mechanism of NFX based on nitroreductase type 1 action.

in adults and 15 mg/kg/day in children, for 60, 90 or 120 days, divided into three daily doses, after meals [1, 68]. It must be highlighted that even at these doses, side effects may potentially be present in adults.

3.4. Safety and efficacy

Many studies regarding the efficacy of NFX were performed between the 1960 and 1980s, at the beginning of its development and first years of commercialization [5, 11, 34]. Considering these studies, the activity of the NFX against the *Trypanosoma sp.* is expressive. NFX is capable to achieve efficacy rates of 70–100% in acute parasitemia cases. However, these rates substantially decrease when it comes to adult chronic phase, reaching values of only 7–8% in adults, although in children under 14, it remains around 86% [68, 69].

The primary reason why BNZ is often preferred compared to NFX is due to the presence of adverse effects. NFX frequently produces side effects [5, 11, 34, 70], but the majority are mild and can be managed with dose reduction or temporary suspension of medication [11, 68, 71].

3.5. Adverse effects

The treatment of CD is long and continuous and the presence of adverse effects becomes quite common between 60 and 100% of the patients [68]. The most frequent side effects described in the case of NFX are anorexia, nausea, headache, and amnesia. The possible neurological reactions are restlessness, disorientation, forgetfulness, insomnia, spasms, paresthesias, polyneuritis, and convulsive seizures. Most of the adverse effects (93%) are mild and disappear through dose reduction or after suspending the treatment [68, 70].

4. Symptomatic treatment of chronic phase

CD is classified evolutionary into two phases: an initial acute phase, followed by a chronic phase. Each phase has distinct clinical characteristics, diagnostic parameters, and treatment [72–76]. The acute phase is characterized by intense parasitism related with nonspecific symptoms, including fever, eyelid edema (denominated Chagoma), edema, and myocarditis. In general, the progression of disease can take years to reach the chronic phase, which presents four clinical forms: indeterminate (without clinical manifestations), cardiac, digestive, and mixed (association of cardiac and digestive) [77–81].

4.1. Chronic Chagas cardiomyopathy (CCC)

Chronic Chagas cardiomyopathy (CCC) is associated with high rates of morbidity and mortality, and it is categorized into stages of cardiac impairment (A, B, C, and D) according to the manifestations, electrocardiograph, radiological alterations, and changes in ventricular function [72, 74, 75, 82, 83]. Approximately 20–40% of patients with the indeterminate form will develop CCC. Several mechanisms contribute to this including the persistence of the parasite and autoimmunity. Moreover, factors such as dysautonomia (neurogenic mechanisms) and microvascular dysfunctions may potentiate and amplify this damage [74, 75, 77, 79–81, 84].

The cardiac form of Chagas can occur with or without ventricular failure [25, 82, 84]. Although the most common is the coexistence of arrhythmic manifestations with the congestive form, some patients have only arrhythmias and intraventricular and atrioventricular conductions compromising with normal ventricular function. Management of CCC treatment is based on the following clinical manifestations such as heart failure, cardiac arrhythmia, and thromboembolism [25, 79, 84].

Although, Chagas disease represents an important cause of heart failure (HF), limited studies have established the use of these drugs in Chagas patients. The treatment of CCC aims to reduce symptoms, delay the evolution of ventricular dysfunction, and prolong survival. In the asymptomatic or mild stages of HF, it is intended to delay the evolution of the disease. In the advanced stages, the objective is to improve the quality of life and the survival of patients. The efficacy and tolerability of these drugs in patients with CCC is extrapolated from the results obtained for other etiologies. Therefore, the CCC treatment is suggested to be performed in accordance with the general guidelines for HF treatment and should consist of the combination of three therapeutic classes: angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB), diuretics, and adrenergic beta blockers (BB) [40, 72, 82].

Angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) have an essential role in adverse cardiac remodeling and ventricular dysfunction progression of HF [40, 72, 82]. In experimental studies reported in the literature, captopril and enalapril demonstrated to diminish myocarditis and fibrosis of CCC [85–88]. These drugs should be administrated initially in low doses. In spite, some physicians report that CCC does not tolerate high doses of ACEI, according to the degree of tolerability; the dose can be progressively increased [25, 78, 81]. ACEI is recommended to patients with a left ventricular ejection fraction (LVEF) <45% [82, 83]. Regarding ARB, spironolactone and eplerenone have been evaluated in studies and data have shown that these drugs are effective in improving the quality of life and reduction of symptoms associated with CCC [89–91] and this class is considered as the treatment of choice for Chagas patients with LVEF <45%, or patients with LVEF<35% and New York Heart Association (NYHA) Class III/IV [82, 83]. In addition, in cases that are contraindicated to ACEI and ARB (hyperkalemia and progressive renal failure), the combination of hydralazine and isosorbide dinitrate should be prescribed [25, 73, 79, 83].

Beta-adrenergic blockers (carvedilol, bisoprolol, or metoprolol) are suggested in association with ACEI and ARB due to autonomous nervous system involvement in CCC and the production of antibodies against β-1 adrenergic and M-2 muscarinic receptors [25, 73, 79, 83]. Limited

reports address the efficacy of this therapeutic class to treat Chagas patients with ventricular dysfunction [86, 92–96]. Beta blockers have been avoided because of the presence of frequent bradycardia; therefore these drugs are not indicated for patients with bradycardia ≤50 bpm or AV conduction disorders (PR > 280 ms) [25, 73, 79, 83].

Moreover, digoxin and diuretics are considered a pharmacological option in CCC, justifying the use of digoxin for patients with symptomatic LVEF ≤45%, principally in the presence of atrial fibrillation when the ventricular frequency is increased and diuretics to improve congestive symptoms and signs. For advanced HF stages, the combination of thiazides with loop diuretics has been proven to be more effective [25, 72, 73, 79, 83].

The annual incidence of thromboembolism in Chagas patients is between 1 and 2%, affecting mainly patients with HF. Occasionally, this aggravation is the first manifestation of the CD. Cardiac emboli can reach both the pulmonary and systemic circulation, with the cerebral territory being the most clinically evident [40, 79, 82]. The treatment of thromboembolism is performed based on the established recommendations, alternating according to the extension and compromised organ. For this, a score derived from a prospective cohort study with 1043 patients was recently available to evaluate the risk and to implement preventing thromboembolisms in CCC. Through risk-benefit analysis, warfarin is indicated for patients with 4–5 point. In the case of 3 points, acetylsalicylic acid (AAS) or warfarin could be used. For 2 points, it is suggested to use AAS or no prophylaxis, and 0–1 points do not need prophylaxis [79, 81, 97].

Patients with CCC usually present ventricular arrhythmia. The most frequent ventricular arrhythmias in Chagas patients are ventricular ectopies, isolated or repetitive. The presence of these arrhythmias in asymptomatic patients with preserved ventricular function does not require antiarrhythmic treatment, whereas in symptomatic cases, the antiarrhythmic treatment can be individualized. The goal of pharmacological treatment of arrhythmias is the control of symptoms. Amiodarone is widely used, despite the high incidence of adverse events. At the usual doses of 200–400 mg/day, it can be associated with alternative agents recommended for cardiopathies of other etiologies, such as propafenone, sotalol, and beta blockers, to reduce severe arrhythmic events. However, drugs belonging to class I (sodium-channel blockers) should be avoided, principally in patients with ventricular dysfunction due to proarrhythmic effects, whereas propafenone is contraindicated in patients with left ventricular dysfunction [77, 79, 83, 98]. Bradyarrhythmia is related with sinus node dysfunction or atrioventricular blocks. For the treatment of symptomatic bradyarrhythmias in CCC, a permanent pacemaker implant is usually performed [98].

4.2. Digestive form of CD

The digestive manifestations of the CD correspond to the functional alterations observed in the esophagus and intestine, which result in the formation of mega-esophagus or megacolon, respectively. These deformations are related with the involvement of the enteric nervous system, especially the Auerbachs plexus. Degenerative phenomena in this region are caused by the presence of inflammatory processes associated with autoimmune responses. Therefore, both megacolon and mega-esophagus results in alterations in motility, and consequently, in slow transit and difficulty to empty, followed by increased organ caliber [99–102].

Mega-esophagus is classified into four groups with the objective of situating the different radiological aspects within the evolutionary spectrum of the affection. In addition, the classification of the mega-esophagus is important for the choice of treatment [99–101, 103].

The symptoms commonly reported in mega-esophagus are: dysphagia, regurgitation and esophageal pain. Treatment of mega-esophagus included clinical, pharmacological measures, dilatation and surgical procedure to aid in the transit of foods and liquids. The choice of treatment to be applied depends on the following factors: patient agreement, relevance of symptoms, degree of classification, nutritional status, clinical condition, comorbidities, age and available hospital infrastructure [40, 103, 104].

Clinical and pharmacological treatment is indicated for patients in group I, or patients at high risk of being treated with other forms of treatment, or in cases refusing invasive treatment. Patients must frequently drink water during the meals, eat slowly, and give preference to food in a pasty consistency. Hot and cold foods and drink and eating prior to bedtime are not recommended because food may be retained in the esophagus, causing pain or nocturnal regurgitation during sleep. The pharmacological treatment is based on substances that aim to relax the esophageal sphincter; however, the beneficial effect of these drugs is restricted to the period of action, being only a symptomatic treatment. The nitric derivatives (isosorbitol dinitrate) and the calcium channel blockers (nifedipine) are recommended [40, 101, 104, 105].

Alternative treatment is forced dilation of the distal segment of the esophagus and esophagic junction using a pneumatic or hydrostatic balloon or surgery. Surgery is performed for patients classified in group II (according to the intensity of the symptoms), III, and IV, and for patients without adequate response to clinical treatment. Another alternative is the injection of botulinum toxin, which acts to inhibit acetylcholine release [40, 101, 105].

The most frequent symptoms in megacolon consist of intestinal constipations, abdominal distension discomfort, occlusive phenomena associated with fecaloma, and sigmoid volvulus [106, 107]. Megacolon treatment may be clinical or surgical and varies according to patient agreement, level of complications, nutritional status, clinical condition, presence of comorbidity, and age [40]. If the clinical treatment indicates an alteration in the diet, use of laxatives, such as lubrificant laxatives (mineral oils) and emollient laxatives, and intestinal washes with water and glycerin may be used [105].

5. Therapeutic alternatives for Chagas disease treatment

5.1. Clinical trials

As previously described in this chapter, a randomized controlled clinical trial for investigation of BNZ in the treatment of CD was performed only recently: the BENEFIT clinical trial [95]. Therefore, it is not surprising that there is a lack of clinical studies with novel drug candidates for CD, since even the study to evaluate the first choice treatment is very recent.

Posaconazole (POS) (**Figure 4A**) is a triazole derivative with antifungal activity [108], that has been approved for the treatment of invasive fungal infections in humans [109]. It has shown trypanocidal activity in murine models [110, 111]. In addition, POS treatment led to the cure of the infection in one patient, resulting in parasite levels lower than after treatment with BNZ [112]. Therefore, POS efficacy and safety were compared to BNZ in phase II clinical trial CHAGASAZOL NCT01162967 [4], since it has shown trypanocidal activity in murine models. In this study, 78 patients were randomly selected to receive POS at a dose of 400 mg, POS at a dose of 100 mg, or BNZ at a dose of 150 mg. All drugs were orally administered twice daily for 60 days. During the treatment days, only two individual of POS patients treated with the dose of 100 mg had tested positive for *T. cruzi* DNA using a RT-PCR assay. The study duration was 1 year, thus the patients were retested following the treatment period. The outcomes have shown a significantly (p-value lower than 0.01) higher incidence of positive *T. cruzi* DNA results in both POS-treated groups (81–92%) compared to BNZ-treated group, since the last one led to only 38% of patients testing positive for *T. cruzi* DNA using the RT-PCR assay. While all patients from POS groups completed the study, five patients stopped the BNZ treatment, due to severe skin reactions. The adverse reactions of the current treatment with BNZ are some of the reasons why superior treatments must be pursued. However, BNZ treatment failed in fewer patients than POS [4].

CHAGASAZOL was not the only clinical trial testing the potential application of POS in Chagas Disease treatment. The clinical trial STOP-CHAGAS NCT01377480 [113] investigated if POS or POS plus BNZ were superior to BNZ monotherapy in the reduction of parasites load after 60 days of treatment and 360 days follow up. In this study, 120 subjects were randomly divided into the following groups: POS 400 mg twice a day; BNZ 200 mg and placebo, both twice a day; BNZ 200 mg and POS 400 mg, both twice a day; or placebo 10 mg. Two outcome stages were considered, the persistence of negative RT-PCR in day 180 and the maintenance of this response by the end of the study in day 360. The successful overall outcome was the RT-PCR negative result in both stages. Both groups receiving BNZ achieve 96% subjects with negative RT-PCR at day 360, while placebo group and POS alone group had 16.7% and 23.3% subjects with negative RT-PCR, respectively. Those groups receiving BNZ also had six serious adverse events reported, such as cutaneous reactions, nervous system disorders, and gastrointestinal symptoms. Therefore once again, BNZ monotherapy was superior to POS, and no advantages were observed in the combination therapy.

Ravuconazole (**Figure 4C**) is another triazole compound that has shown potent and specific anti-*T. cruzi* action *in vitro*, but with limited *in vivo* activity, probably as a consequence of its poor pharmacokinetic properties [114]. Therefore, E1224 (**Figure 4D**) was developed as a prodrug of ravuconazole. A phase II Clinical Trial was performed in 2013 to evaluate the prodrug efficacy and safety compared to BNZ, as a randomized double-blinded placebo-controlled proof-of-concept study [115]. The study was performed in 224 patients with chronic indeterminate CD. Two groups received E1224 for 60 days at doses of 200 mg (low dose) and 400 mg (high dose), one group took E1224 for 30 days at a dose of 400 mg (short dose), and one group received BNZ at dose of 5 mg/kg/day. All treatments were able to eliminate blood-circulating parasites during the treatment period. E1224 had a parasite clearance rate of 90, 89, and 76% for the low dose group, short dose group, and high dose group, respectively. However, at the 12-month evaluation clearance of all patients that received E1224 was significantly low, and only the high dose group (29%) led to results significantly better than placebo.

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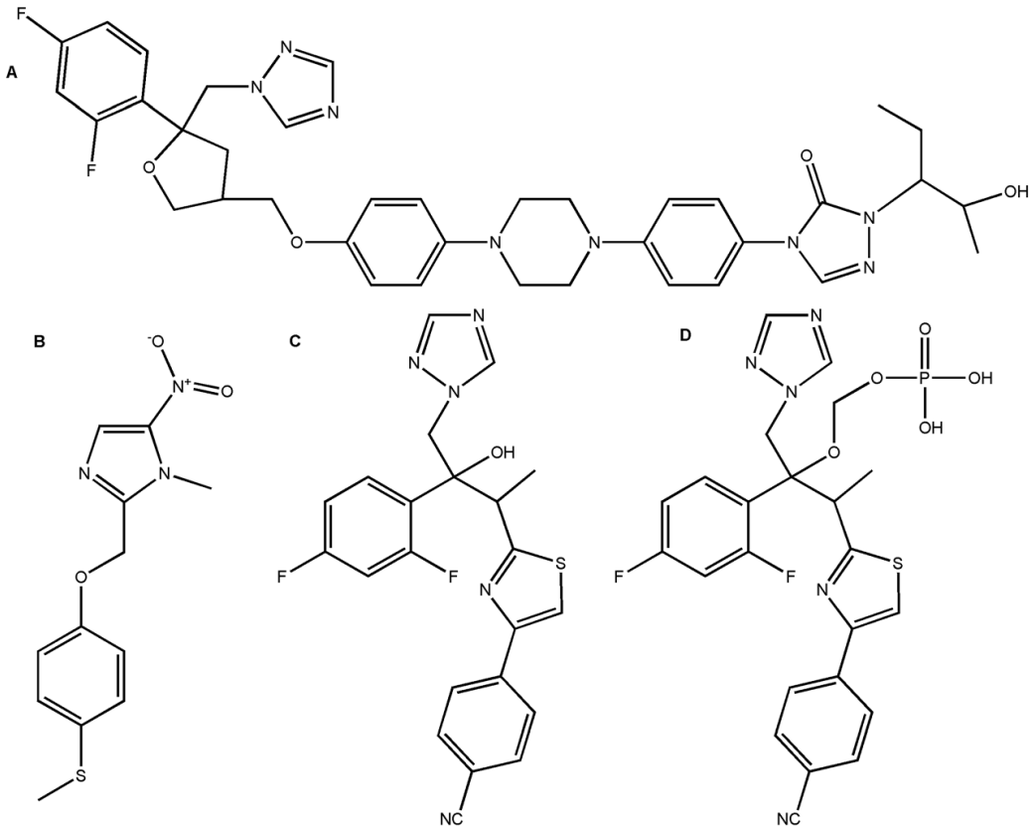

Figure 4. Structures of the compounds posaconazole (A), fexinidazole (B), ravuconazole (C), and E1224/fosravuconazole (D).

Alternatively, the BNZ clearance was 91% at 60 days after treatment and it dropped to 82% at 12 months, also being significant versus placebo. In summary, the drug candidate E1224 was as effective as BNZ at clearing the parasite following the treatment course, but the sustained efficacy 1 year following treatment was very low. Further, BNZ led to significant side effects. Therefore E1224, now called fosravuconazole, will be clinically tested in association to BNZ in order to improve treatment tolerability issues by combining them in the disease therapy.

A search for CD studies of new treatments on the clinical trials website (clinicaltrials.gov) provides one more study, with fexinidazole (**Figure 4B**). This compound is an old nitroimidazole that has shown promising results in preclinical models of CD [116]. According to Drug for Neglected Disease initiative (DNDi), this clinical study NCT02498782 was stopped due to safety and tolerability issues that occurred at the highest tested dose. High efficacy was observed at the lowest dose with acceptable safety and tolerability. Based on this, DNDi claims to be planning a new study in the near future [117].

5.2. Preclinical studies

Considering the lack of new and safer treatment options for CD, it is very encouraging to observe the efforts of many research groups investigating suitable candidates, which may improve the quality of life of many Chagas disease patients. Among the proposed targets for new alternative therapies examples are, ergosterol synthesis, cruzipain, trypanothione reductase, and type I

nitroreductase. Studies addressing these targets in several phases of development will be discussed in this text. However, only a few compounds have demonstrated *in vivo* activity and favorable disposition, while none of the published preclinical candidates seems to be in the boundary of the clinical studies.

5.2.1. CYP51

The sterol 14α-demethylase (CYP51) is an important enzyme for *T. cruzi* survival [118], which works as a target of broad-spectrum antifungal agents, such as POS and ravuconazole, or its prodrug fosravuconazole [119]. Since they were clinically tested, as previously mentioned, they are the most close that a new treatment has come to the patients, even though the clinical studies outcomes were not the best [113]. Albaconazole was considered promising after *in vitro* and *in vivo* studies, but its effect was not satisfactory against a resistant strain [120, 121].

Several others inhibitors of sterol biosynthesis have been tested in non-clinical phases. TAK-187 is another antifungal agent [122] tested for CD due to its good drug-like properties and mechanism of action aiming at CYP51. It has shown potent anti-*T. cruzi* activity *in vitro* and *in vivo* [122], while preventing *Trypanosoma cruzi*-induced cardiac damage [123]. Recently, a new formulation of TAK-187 was patented [124], indicating that a clinical study is potentially possible.

Dialkyl imidazoles have shown high potency (EC50 in the 0.4–10 nM range) against *T. cruzi in vitro*, leading two of them to be tested *in vivo* [125]. These derivatives were able to reduce the parasitemia to undetectable levels in a mouse model of acute CD. According to the authors, these compounds are less expensive to be produced than POS. Carrying systematic modifications in the proposed scaffold of the aforementioned candidates led to new candidates with an EC50 better than that of POS (1 nM); however, only *in vitro* studies have been performed [126].

Since tipifarnib has shown potent anti-*T. cruzi* activity, it is being considered as a new lead compound for CD treatment [127]. To evaluate this drug, analogs of the antitumor agent were designed and investigated *in vitro*. They were considered advantageous due to their modest potency for inhibition of human CYP3A4 [128]. One of these analogs, compound 2 was evaluated *in vivo*, but was not better than POS or BNZ [129]. Lack of adequate pharmacokinetic properties, with short half-life and low exposure, may cause the tipifarnib analog to be ineffective.

A very rational study was performed in the development of 4-aminopyridyl-based CYP51 inhibitors [130]. In this study, compounds were developed and tested for their *in vitro* activity. To assess their action *in vivo*, the pharmacokinetic study was previously performed to better understand the compounds disposition and modify the vehicle of administration. Some of the derivatives have demonstrated better *in vivo* activity when compared to the *in vitro* studies. For the authors, the disconnection between *in vitro* and *in vivo* potency raised questions about prioritizing the CYP inhibitors based only on their *in vitro* performance. They achieved lead compounds with good potency and oral bioavailability, while they admit the need for optimization of the elimination half-lives.

A similar study design was followed to evaluate analogs of fenarimol [131], where it was observed that compounds with long half-lives, that is, maintaining plasma concentration above the IC50, were those that led to undetectable parasite levels in bloodstream. The

compounds that were less effective to reduce the parasitemia were those with fast elimination and plasma concentrations equals or below the IC50. The two lead compounds were highly bioavailable with a long half-life. The *in vivo* efficacy was superior compared to BNZ and comparable to POS.

Assuming an evolution of the antifungal approach, VNI is an imidazol developed to be specifically active in the inhibition of the CYP51 of *T. cruzi*. It was able to cure the acute and chronic forms of CD in mice, with 100% survival and no observable side effects. Its pharmacokinetics were considered suitable for further development; however, the presented data were not completely explored [132]. The VNI optimization, VFV, overcame the promising results of VNI not only in the animal model of CD cure rate, but also with better pharmacokinetic properties and more information regarding distribution and biotransformation [133]. The reporting of VFV acute toxicity study have shown no observed adverse effect up to 200 mg/kg in mice, but authors did not present data to assure that the absorption was not saturated, that is, the exposure was proportional to the doses used in the acute toxicity study [134]. These imidazole-containing compounds seem to be the most advanced compounds, but the authors highlight the need for further studies.

5.2.2. Cruzipain

The cysteine proteinase cruzipain is vital for *T. cruzi* survival, since it is essential for the parasite replication and differentiation. Based on this, cruzipain has been considered as a target for drug discovery, but also for vaccines [135]. Phe-ala-PQ was a prodrug of primaquine, which had the intent to use the proteinase function of cruzipain to release the parent molecule in the parasite [136], but there are others.

Vinyl-sulfone derivatives [137], such as K11777 and WRR-483 have irreversible inhibitory activity in cruzipain, leading them to be effective against *T. cruzi in vitro* and, also *in vivo* [138, 139]. On the other hand, Cz007 and Cz008 are vinyl-sulfones containing nitrile with reversible cysteine proteinase activity that showed *in vivo* anti-*T. cruzi* action following oral administration in a dose lower than that of BNZ [140, 141]. Authors report bioavailability of both new compounds to be approximately 50%, but with short elimination half-life, what led them to administer the compounds for the efficacy study mixed to powdered chow, while BNZ was administered in water.

Additional compounds focusing on cruzipain have their anti-trypanocidal activity published only *in vitro*, such as thiazolyl hydrazones [142], thio semicarbazones [143], acylhydrazones [144], dipeptidyl nitrile derivatives [145], and triazine and purine nitriles [146].

5.2.3. Trypanothione reductase

Trypanothione reductase is responsible for the parasite antioxidant protective mechanism, thus inhibitors aiming for this target may disrupt its antioxidant defenses [147]. Some tricyclic compounds already active for other conditions were able to inhibit trypanothione reductase, and clomipramine was the most active with an inhibition constant (Ki) of 6 μM [148]. The testing for new active compounds led to the discovery of inhibitors with a Ki up to 0.33 μM

[149]. Among the phenothiazines, thioridazine is one of the most potent irreversible inhibitors of trypanothione reductase [150], and has shown better *in vivo* activity than the non-treated group [151]. When compared to BNZ, thioridazine animal survival was comparable of BNZ in mice infected with *T. cruzi* [152].

Moreover the trypanothione reductase inhibition, hydroxymethyl nitrofurazone (NFOH) is able to inhibit cruzipain by 60%, showing *in vivo* activity and experimental animal survival comparable to BNZ [153]. The NFOH pharmacokinetics were studied in rats and rabbits [154, 155], and indicated that optimization is needed despite the dual action.

Additional compounds able to inhibit trypanothione reductase *in vitro* include quaternary analogs [156] of chlorpromazine. The dibenzosuberyl-containing analogs of clomipramine were poor inhibitors of trypanothione reductase, while the polyamine derivatives containing dibenzosuberyl were potent inhibitors up to 0.26 μM, even though they were not active *in vivo*, maybe due to poor pharmacokinetic properties, which were not evaluated [157]. Studying the *in vitro* anti-*T. cruzi* activities of ethyl and methyl quinoxaline-7-carboxylate 1,4-di-N-oxide derivatives, three compounds were discovered. However, only one was non-cytotoxic on host macrophage cells [158], and no *in vivo* study was presented.

5.2.4. Type I nitroreductase

Type I nitroreductase is an enzyme responsible for the differentiation of *T. cruzi* and for the activation of nitroheterocycles [159]. Nitroreductases are among the enzymes proposed as responsible for the bioactivation of BNZ [159] and NFX [160]. Based on this hypothesis, the impairment of this enzyme would be responsible for resistance [159].

The type I nitroreductase bioactivation approach supports the *in vitro* action of aziridinyl nitrobenzamide [161] and halogenated nitrobenzylphosphoramide mustards [162] prodrugs; however, the last was assessed in *T. brucei* parasites. Several nitrotriazole-based compounds demonstrated significant *in vitro* antichagasic activity [163, 164]. Further studies identified compounds with promising *in vivo* activity that has shown good *in vitro* ADME properties [165].

Nitrotriazole compounds with dual action aiming type I nitroreductase and inhibition of CYP51 were able to clear the parasites following a 10-day treatment, better than what was observed in the previous mentioned monofunctional 3-nitrotriazole-based derivatives [166]. Prior to the positive results of the dual action compounds, some experiments led to the understanding that nitroheterocylic compounds are better than those able to inhibit ergosterol biosynthesis [167], while it seems that together, they can work better.

Many preclinical candidates have only proven to be effective *in silico* or *in vitro* becoming promising leads or proving the validity of the proposed strategy. Novel drug candidates with *in vivo* activity, in limited cases have published disposition data, such as bioavailability and elimination half-life. The crucial study before clinical studies is the achievement of the NOAEL, since it is necessary to calculate the first in human dose. However, for compounds not showing clear toxicity without the pharmacokinetic assessment, the NOAEL can be overestimated due to saturated absorption, and this can be an challenge for compounds without this data [168].

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5.2.5. Combined alternatives in preclinical studies

Based on the safety issues of BNZ and NFX, strategies to reduce their administered amount would prove beneficial as it may decrease the adverse effects. To circumvent the decrease in efficacy due to dose reduction, combined therapies have been proposed.

The combination of the diamidine prodrug DB289 and BNZ orally decreased parasitemia by 99%, while alone they led to 70 and 90% for DB289 and BNZ, respectively. When the combination of BNZ and DB766 was administered, the decrease in the parasitemia was at least 99.5%. Both combined treatments provided 100% protection against mice mortality, while BNZ alone provided only 78% protection [169].

The combination of BNZ and ketoconazole were evaluated in a disease mouse model. This led to better results than single treatments with susceptible and moderately resistant (Y) strains of *T. cruzi*. [170]. The same Y strain of *T. cruzi in vivo* was used to evaluate the combination of BNZ and itraconazole and the results indicated a fourfold improvement in the disease outcome against each single treatment. Additionally, this combination led to a decrease in cardiotoxicity in the experimental CD [171].

The new compound tetrahydro-β-carboline N-butyl-1-(4-dimethylamino)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxamide was superior among its peers in reducing the number of infected cells and the number of internalized parasites, with low cytotoxicity [172]. After this observation, the selected compound was evaluated in combination with BNZ *in vitro* and *in vivo*, where a synergic effect was observed, leading to a reduction in parasitemia and mortality rates [173].

An additional compound tested with BNZ was the new drug candidate 2-methyl-1,5-bis(4-nitrophenyl)penta-1,4-dien-3-one, which has shown trypanocidal activity *in vitro*. Next, it was evaluated in combination with BNZ, ketoconazole, and fluconazole. Although the combination with the last one was ineffective, the combination with BNZ and ketoconazole demonstrated strong synergism [174].

These studies indicate that combination of new compounds or compounds used for alternative conditions have the potential to be a successful approach. A similar strategy has been planned for clinical trials to evaluate the combination of benznidazole and fosravuconazole, despite the absence of advantage in the combined therapy using BNZ and POS.

6. Conclusion

Once no new therapeutic alternatives have been included in its treatment portfolio after benznidazole and nifurtimox introduction there is a need for new compounds able to treat patients of CD. Many new drug candidates are evaluated to provide a better treatment for patients in the chronic phase. Benznidazole and nifurtimox are very effective, but their adverse effects cause many patients to discontinue treatment. Symptomatic treatment of the chronic phase of the disease is as important as the treatment directed in the reduction of the parasite. It

is necessary to evaluate the patient conditions correctly to improve the quality of life. It is comprehensible that the chase for new options against this disease begins with a search of new molecules, but it is important to keep in mind that this is just a small step. Significance can only be measured if the following steps are performed and the patient treatment is reached. The combined therapy and the repurposing approaches may discover the next treatment option; however, the research must not be completed until the ideal treatment is achieved.

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