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FOR SUCH AN ANCIENT DISEASE, A GLIMPSE OF PROMISING NEW TREATMENTS FOR CHAGAS DISEASE

PARA UMA DOENÇA TÃO ANTIGA, O VISLUMBRE DE NOVOS TRATAMENTOS PROMISSORES NA DOENÇA DE CHAGAS

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ABSTRACT

It has been more than 100 years since the discovery of Chagas Disease (CD). However, the repertoire indicated for its treatment is still limited. Thus, this article aims to present a review of the new pharmacological strategies being studied for CD. This literature review, consisting of 68 articles, from 1957 to 2021, was carried out on several scientific platforms. Positive effects from benznidazole have been described in the acute and chronic phases, in addition to its association with itraconazole in the acute phase. Among the cruzain inhibitors, the compound K777 presented trypanocidal effects, although demonstrating major adverse effects, while its analogue WRR-483 demonstrated great beneficial effects in vivo and in vitro. As for the nitroheterocyclics, fexinidazole showed high rates of cure in animal model, in addition to low toxicity. Nifurtimox, in early chronic stages, was able to delay the progression of tissue damage and reduce the parasite load. The compound WC-9, a squalene synthase inhibitor, showed potential inhibition of *T. cruzi* replication. Regarding aromatic diamidines, many compounds were able to stop the trypanosome, both in vitro and in vivo models. It was concluded that there are favorable findings to improve the treatment of CD. However, the development of effective new drugs does not only depend on their effective action, but also on numerous variables that must be circumvented, such as the reduction of side effects, treatment time and adherence to the current medication of choice, as well as the investment in production and distribution to the population.

Keywords: Chagas disease. Innovatory. Treatment. *Trypanosoma cruzi.*

RESUMO

Há mais de 100 anos desde a descoberta da Doença de Chagas (DC). No entanto, o repertório indicado para o tratamento ainda é limitado. Dessa forma, este artigo visa a apresentar uma revisão das novas estratégias farmacológicas em estudo para a DC. Esta revisão de literatura, composta por 68 artigos, de 1957 a 2021, foi realizada em várias plataformas científicas. Foram descritos efeitos positivos do benznidazol, na fase aguda e crônica, além de sua associação com itraconazol na fase aguda. Dentre os inibidores de cruzaína, o composto K777 apresentou notório efeito tripanocida, apresentando, no entanto, efeitos adversos importantes, além de seu análogo WRR-483, o qual demonstrou efeito benéficos *in vivo* e *in vitro*. Quanto aos nitroheterocíclicos, o fexinidazol apresentou altos índices de cura em modelo animal, além de baixa toxicidade. Já o nifurtimox, em fases crônicas iniciais, foi capaz de atrasar a progressão de lesões teciduais e diminuir a carga parasitária. O composto WC-9, inibidor da esqualeno sintase, demonstrou potencial inibição na replicação do *T. cruzi*. A respeito das diamidinas aromáticas, inúmeros compostos foram capazes de frear o tripanossoma, tanto em modelos *in vitro* quanto *in vivo*. Concluiu-se que há descobertas favoráveis à melhoria no tratamento da DC. Todavia, o desenvolvimento de medicamentos efetivos não depende somente de sua ação eficaz, mas de inúmeras variáveis que devem ser contornadas, como a diminuição de efeitos colaterais, tempo de tratamento e adesão da atual medicação de escolha, ou mesmo o investimento na produção e distribuição da mesma à população.

Palavras-chave: Doença de Chagas. Inovador. Tratamento. *Trypanosoma cruzi*.

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INTRODUCTION

In 1909, Carlos Justiniano Ribeiro Chagas, a Brazilian public health doctor, made history due to his discovery of Chagas Disease (CD) based on his studies in Lassance, a small town in the interior of Minas Gerais (MALAFAIA; RODRIGUES, 2010). This is a historic milestone in the medical scientific community, as Carlos Chagas was one of the few scientists to discover a pathology, describe the causative agent of the disease, *Trypanosoma cruzi*, identify the transmitting vector, the hematophagous triatomine popularly known as the "barber", and produce a dissertation on the epidemiological, clinical and social data of his new discovery (MAGALHÃES, 2010).

Currently, CD is endemic in 21 countries in the Americas and affects approximately 6 million people. It is estimated that there are 70 million people living in areas of exposure and risk in American countries, with an incidence rate of 30,000 cases per year and an annual number of deaths close to 14,000 (ORGANIZACIÓN PANAMERICANA DE LA SALUD, 2017). In Brazil, measures taken regarding vector control, screening of candidates for blood, organ and tissue donation substantially reduced the incidence rate of CD cases in the Brazilian territory (BRASIL, 2019). However, from 2005 onwards, mandatory notification was resumed on account of the repercussions on national public health due to the identification of oral transmission of the disease (DIAS *et al*., 2016).

CD has two well-established clinical phases: acute and chronic. The first is characterized by the presence of the metacyclic trypomastigote form of *Trypanosoma cruzi* in the blood, responsible for the first contact with the host cell, which depends on the interaction of molecules of the parasite with the cell, a series of cascade signaling and Ca^{2+} mobilization, in order for the cell invasion process to occur (YOSHIDA; CORTEZ, 2008). Acute symptomatology, when present, is expressed by malaise, fever, hepatosplenomegaly, lymph node infarction, cardiac disorders or even the presence of Romaña's sign, unilateral eyelid edema caused by contamination of the ocular conjunctiva by infected triatomine feces (KÖBERLE, 1968).

After this stage, the disease enters a long asymptomatic period, in which the presence of the parasite in the bloodstream is at undetectable levels. The evolution of the disease culminates in the chronic phase, in which the manifestation of the cardiopathy, digestive or associated (cardiodigestive) form can be observed (DIAS; COURA, 1997). The chronic phase is the result of the proliferation of the parasite in the muscle fibers of the heart, esophagus or colon, during the asymptomatic period, which leads to generalized inflammatory processes in the infected tissues, resulting in severe dysfunction of affected organs (LOPES; CHAPADEIRO, 1997). Cardiac heart disease can be fatal, its outcome is related to the increase in the heart and rhythm disturbances, culminating in congestive heart failure and increased risk of sudden death (ROSSI, 1990).

It has been over a hundred and ten years since the discovery of CD, and despite so much time of study, development and investment, the drugs on the market indicated for the treatment are still restricted to little variability and availability compared to others zoonoses. However, recent studies have shown promising results in the formulation of new therapies that promise to help in the treatment. This article aims to present a review of the newest pharmacological strategies being studied with promising results in the treatment of CD, focusing on the targets of these compounds.

MATERIAL AND METHODS

A literature review was carried out, looking for information on the development of new therapeutic regimens used in the treatment of CD and their applicability in terms of cost-effectiveness. The search was carried out using the following digital platforms: USP Digital Library; Scielo; Google Academic; PubMed; EBSCO; CAPES and ScienceDirect. Terms used in these platforms were: "Doença de Chagas", "novos tratamentos", "Benznidazol", "Diamidinas aromáticas", "Antifúngicos Azólicos", "Benzonidazol Itraconazol", "Inibidor de Cruzaína", "Nitroheterocíclicos", "Inibidores de Esqualeno Sintase" and the corresponding English translations "Chagas Disease", "new treatments", "Benznidazole", "Aromatic Diamidines", "Azole Antifungals", "Benznidazole Itraconazole",

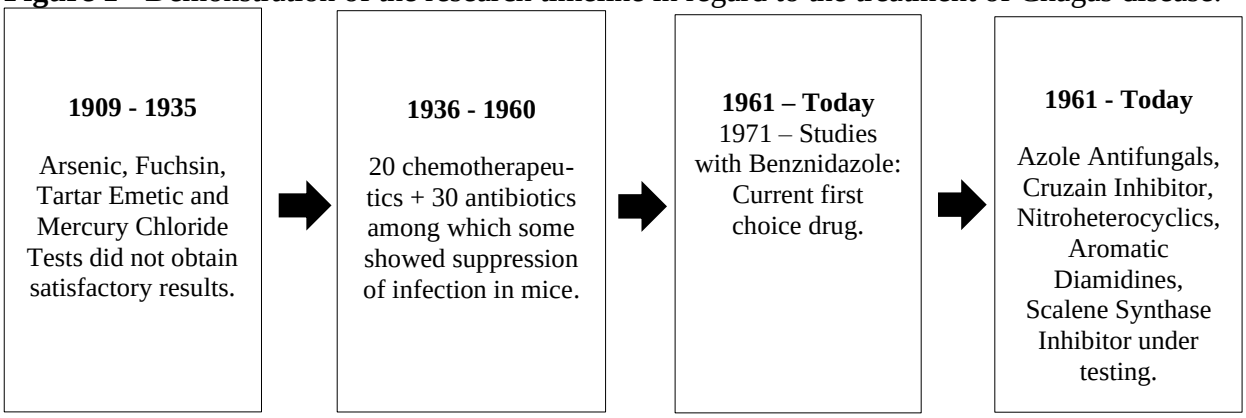
"Cruzaine Inhibitor", "Nitroheterocyclics", and "Squalene Synthase Inhibitors". A total of 68 articles were selected, according to the pre-defined inclusion criteria, all of which were published between 1957 and 2021.

RESULTS AND DISCUSSION

Treatment History

The evolution of CD treatment can be divided into three long periods. The first is between 1909 and 1935, in which two studies were published about the arsenic, fuchsine, tartar emetic and mercury chloride test, which did not obtain satisfactory results (DAVANÇO, 2015). From 1936 to 1960, there was a greater number of published works, in which results were described for about 20 chemotherapy agents and 30 antibiotics, with more promising outcomes for compounds (DIAS *et al*., 2009), such as nitrofurazone, furaltadone and furazolidone, which acted in the suppression of trypanosome infection in mice (PACKCHANIAN, 1957).The third period runs from 1961 to the present day, a phase in which there is the largest number of studies and evaluations of substances in experimental models. During this period, the compounds lapachol, β-lapachol and their derivatives have been described as substances with trypanocide potential from the induction of free radicals release, providing a change in the chromatin structure, swelling in the mitochondrial membrane, in addition to inhibiting DNA and RNA synthesis, among other changes in protein synthesis, and cytoplasmic and nuclear membrane (PINTO; CASTRO, 2009). However, it was demonstrated that these substances could be inactivated through interaction with serum proteins or by contact with oxyhemoglobin (LOPES *et al*., 1978). Around 1971, two drugs appeared with positive results in the acute phase: benznidazole and nifurtimox (DIAS *et al*., 2009). Currently, benznidazole (BNZ) is the drug of choice in the treatment of acute CD (DIAS *et al*., 2016).

Figure 1 - Demonstration of the research timeline in regard to the treatment of Chagas disease.



Source: Dias *et al.* (2009), Davanço (2015) and Dias *et al.* (2016), adapted.

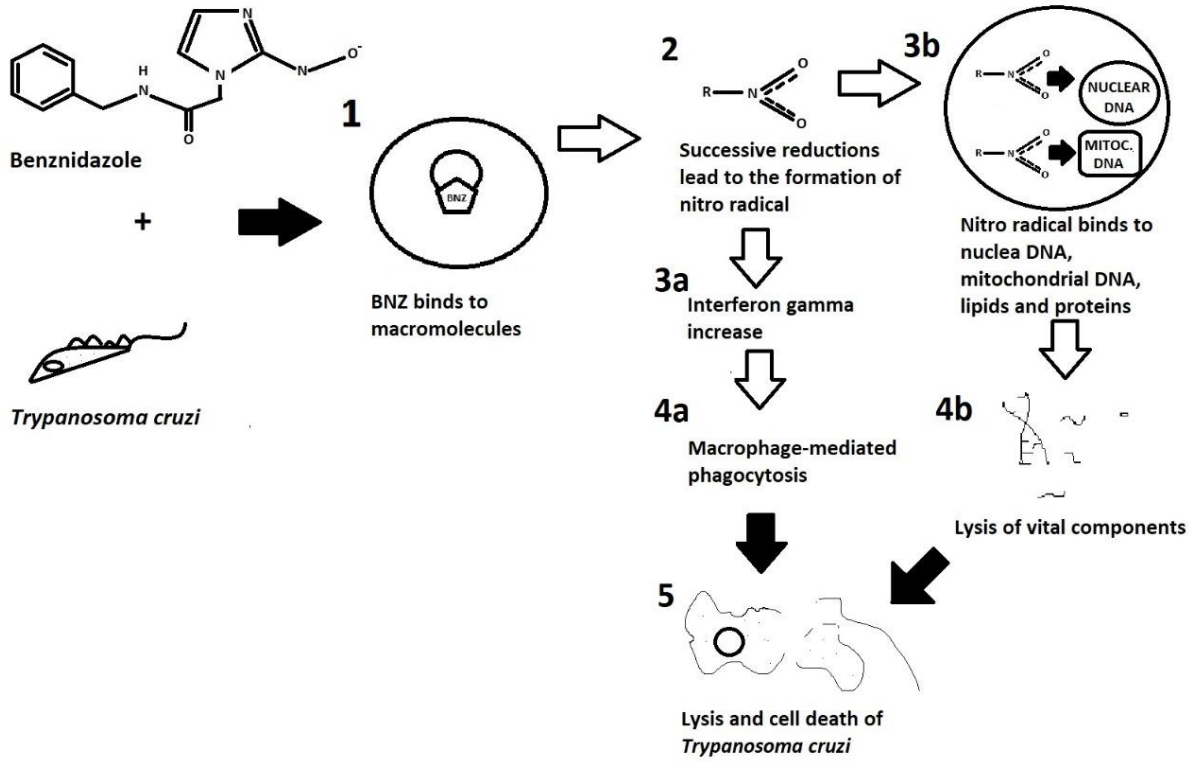
Benznidazole

Used as a first-line treatment in Brazil, Benznidazole (BNZ) exerts an effect by binding to parasite macromolecules. This occurs through metabolizations, described in Figure 1 (CASTRO; DIAZ DE TORANZO, 1988; DAVANÇO, 2015), in addition to cell signaling in the synthesis of IFN-γ, which induces phagocytic cells to produce reactive oxygen and nitrogen species, also activating CD8+ T lymphocytes, which start to perform their cytotoxic activity (LOURDES, 2013; ALEXANDRE; TESTON; ZANUSSO JÚNIOR, 2014). In this way, it reduces parasitemia, leading to cure in the acute phase in most cases (COURA; BORGES-PEREIRA, 2012).

Some current studies also suggest that treatment in the chronic phase can reduce the consequent affections caused by the trypanosome, with indices that point to cardiac preservation, less dyspnea and an increase in the serum concentration of interleukin-17 (IL-17) mediated by BNZ. IL-17 is an immunoregulatory interleukin essential for systemic protection in the chronic phase of Chagas (CAMARA *et al*., 2018). Recently, Cardoso and contributors (2018) demonstrated that when starting treatment with BNZ in patients with chronic CD, aged under 50 years and demonstrating electrocardiogram changes, there was a significant reduction in mortality, parasite load and degree of heart failure, when compared to the control group that had not received BNZ after two years of follow-up (CARDOSO *et al*., 2018). Recently, Cardoso and contributors, demonstrated that when starting treatment with BNZ in patients with chronic CD, aged under 50 years and with changes in the electrocardiogram, there was a significant reduction in mortality, parasite load and degree of heart failure, when compared to the control group that had not received BNZ after two years of follow-up (CARDOSO *et al*., 2018). However, the use of BNZ is contraindicated in the late phase of chronic heart disease (in which there are severe arrhythmias, ejection fraction < 40% or presence of heart failure) induced by CD, as published by the Ministry of Health (CONITEC, 2018). This is because in the chronic cardiac phase it has not been shown that BNZ is capable of slowing the progression of heart disease, and it may even worsen the condition (VIOTTI *et al*., 2006; MORILLO *et al*., 2015).

Currently, in addition to the 100 mg formulation for adults, there is also a pediatric formulation. Since 2011, 12.5 mg tablets, used for babies and children up to two years old suffering from Chagas disease, have been approved. The recommended dosage is 5 to 7 mg/kg daily, divided into two doses. In addition to the favorable dosage, the tablets can be easily disintegrated in drinking water or orange juice, facilitating their administration to the indicated age group (DNDI, 2011; LAFEPE, 2018).

Figure 2 – Schematic of the mechanism of action of Benznidazole against *Trypanosoma cruzi*



Source: Castro, Dias de Toranzo (1988), Coura, Borges-Pereira (2012) and Davanço (2015), adapted.

Azole antifungals: Itraconazole and Posaconazole

In addition to the vital macromolecules on which BNZ acts, another biochemical target capable of disabling *T. cruzi* activity is the pathway that synthesizes ergosterol. It is a vital component in the structure of the parasite's plasma membrane, whose function is similar to that of cholesterol in human membranes (FIUZA, 2018). This class of drugs – azole antifungals – act by inhibiting the CYP51 enzyme, in order to prevent lanosterol from being converted to zymosterol and later ergosterol, culminating in an afunctional plasma membrane (CATALÁN; MONTEJO, 2006). In addition to preventing the formation of this steroid, drugs in this class also ensure that toxic precursors accumulate in the cell membrane, affecting its integrity in two ways (SUETH-SANTIAGO *et al*., 2015). Separately, in vitro tests were carried out with fluconazole, itraconazole (ITZ) and ketoconazole, drugs whose antifungal activities are proven. However, contrary to expectations, they did not achieve significant results in combating protists in vivo in the acute phase of Chagas disease, by not reducing the systemic parasite load to sufficient levels (COURA; BORGES-PEREIRA, 2012; SUETH-SANTIAGO *et al*., 2015). This can be explained by the lipophilicity of ITZ: good oral absorption does not occur, generating low bioavailability. In addition, the drug has low potency, requiring high doses to achieve the desired effect in vivo against the trypanosome (FRANÇA *et al*., 2014).

As for tests in the chronic phase, an experimental study inoculated trypanosome strains in 120 mice and kept them alive without treatment for 90 days, in order to chronify the disease. At the end of the three months, only 60 mice survived and these were then divided into three groups. The first group had 45 animals that received 100mg/kg/day, in a single daily administration through a gastric tube for 90 days. In the second group, 15 infected animals remained untreated, the control group undergoing a natural evolution of the disease. The third group consisted of 20 uninfected animals. The results showed that there were only 22 survivors in the first group, compared to 11 in the second group and 9 in the third. 20 mice from the first group showed histopathology with chronic inflammation, compared to 9 from the second group. It was concluded, therefore, that the treatment was also not satisfactory in the chronic phase of the disease (MOREIRA *et al*., 1992).

Regarding adverse reactions caused by ITZ, patients with heart disease have contraindications due to drug-induced cardiotoxicity, which can trigger heart failure or cause progression of heart disease, if it already exists before using the drug. The pathophysiology of this change has not yet been elucidated (PAUL; RAWAL, 2017). In patients without a history of previous heart disease, doses above 400mg can also lead to the same adversity. Furthermore, as ITZ inhibits 14-a-desmethyls (hepatic cytochrome P450 enzyme (CYP51A), mentioned above), it can cause drug interactions with drugs that depend on this enzyme for metabolization (KURN; WADHWA, 2020).

Another azole antifungal tested for trypanosome eradication capacity was posaconazole (PNZ). Derived from ITZ, PNZ has been reformulated into a more hydrophilic molecule, ensuring better oral absorption. Furthermore, it kept the active principle of ITZ, inhibiting the CYP-51 enzyme and preventing the formation of ergosterol (FRANÇA *et al*., 2014). A prospective cohort study carried out in Spain in 2014 with 78 patients with CD in both the chronic and acute phases, duly randomized, without previous treatment, to investigate the antifungal's trypanocide capacity. Patients were divided equally into three groups that received, respectively, oral PNZ twice daily at the dose of 100 mg, oral PNZ twice daily at the dose of 400 mg, and oral BNZ twice daily at the dose of 150 mg. After the end of the 60 days of treatment, the participants were still followed up for 40 weeks to perform serological tests. The first results collected were promising: after 14 days of treatment, the tests performed showed a decrease in the protozoa in the patients' bodies. However, during the next 40 weeks, the protozoan was identified again: through RT-PCR (real-time polymerase chain reaction), there was positivity for *T. cruzi* in 92% of the members of the first group (PNZ at low dose), 81% in the second group (high-dose PNZ) and only 38% in the third group (BNZ). Patients in the low-dose PNZ group were the first to experience treatment failure, obtaining a positive result at the end of 60 days of treatment (MOLINA *et al*., 2014). The main adverse symptoms observed in patients who used

PNZ were: neutropenia, anorexia, dizziness, headache, paresthesia, drowsiness, abdominal pain, elevation of liver enzymes (TGO, TGP, alkaline phosphatase, Gamma GT), rash, asthenia and fever (SCHERING -PLOUGH, 2020).

Benznidazole associated with Itraconazole

A trial carried out in Ouro Preto, at the Chagas Disease Laboratory, sought to assess the association of BNZ and ITZ in combating acute infection. For this, young dogs inoculated with the *T. cruzi* strain were used, distributed into four groups that were treated respectively, only BNZ, only ITZ, BNZ associated with ITZ, and the fourth group was not treated. The results showed therapeutic success in all groups, being 50% in the case of dogs treated in group 2 (ITZ), 60% in group 3 (BNZ and ITZ), and 77% in group 1 (BNZ). Although all groups showed a decrease in *T. cruzi* load, one animal in group 3 (BNZ and ITZ) was considered fully cured, according to the most current evaluation criteria, as it showed negative results in all tests performed, including ELISA, taken as the gold standard in the assessment of cure after treatment. In addition to this, two other dogs, one from group 2 (ITZ) and the other also from group 3 (BNZ and ITZ), were also considered cured according to alternative healing criteria, in which parasitological methods are considered, such as fresh blood test, trypomastigote concentration, xenodiagnosis and blood culture (LANA; MARTINS-FILHO, 2015; CUNHA, 2017).

The ability of the association of BNZ and ITZ to reduce the parasite load and/or achieve cure in mice treated in the acute phase of infection has already been demonstrated. In addition, the tissues of mice that were not cured and that reached the chronic phase were also analyzed, with histopathology demonstrating less inflammatory damage when compared to the untreated control group or those treated with only one of the two drugs (SILVA, *et al*., 2015). It is undeniable that this trypanocide potential is due to the synergy existing between the drugs due to the difference in the mechanisms of action, which interfere in the survival of *T. cruzi* through two inhibition pathways. However, there is also a pharmacokinetic interaction capable of prolonging the half-life of BNZ, increasing its bioavailability. ITZ also works, therefore, as an enhancer of BNZ, with even more benefits in administering both simultaneously (SILVA, 2011).

Cruzain Inhibitor: K777 and WRR-483

Cruzaine is the most abundant cysteine protease in *T. cruzi*, essential for parasite differentiation and development. Among the main substances involved in inhibition, fluoromethylketones, triazoles, pyrimidines, thiosemicarbazones, chalcones, benzimidazoles and vinylsulfones stand out. The latter stood out for its potent activity demonstrated in in vitro and in vivo assays, due to its high affinity for the catalytic site and high affinity for the target enzyme, in addition to its effectiveness in models tested in animals (LEITE, 2013). Therefore, the most studied drug in this class is the compound K777 or K11777, which irreversibly binds to cruzain through the nucleophilic addition of catalytic cysteine to the vinyl sulfone group present in its structure (REYS, 2019).

On the other hand, the mentioned irreversible binding to cruzain generates as an adverse effect hypothermia when binding to homologous cysteine proteases present in the mammalian body, in this case the C3H mice (ENGEL *et al*., 1998). Furthermore, an important drug-induced hepatotoxicity was also discovered. K777 causes a significant elevation of alanine aminotransferase (ALT), indicating liver tissue damage, which kept the study restricted to the pre-clinical phase. Furthermore, the results of this study showed that the dose estimated to be hypothetically consumed by humans would be an oral dose of 10 mg/kg for 14 to 30 days, a dose in which the aforementioned side effects would be present (GARCÍA-TORRES; PÉREZ-MONTFORT, 2011). Another drug in this class studied was the compound WRR-483. It is a K11777 analogue, whose phenylalanine substituent (the substance responsible for interacting with the active site of the cruzain enzyme) was replaced by the

arginine substituent. WRR-483 proved to be a more effective cruzain inhibitor in cell assays in vitro in cultures of *T. cruzi* and in vivo in mice than the compound K11777 (WIGGERS, 2011). However, the pharmacokinetic properties of the analogue WRR-483 are not favorable, as it does not have oral bioavailability, researches seek to develop a derivative compound with equal potential and characteristics that facilitate its administration, however, this objective has not yet been reached (JONES *et al*., 2015).

Nitroheterocyclics: Nifurtimox and Fexinidazole

Nitroheterocyclics are the current drug class of choice used in the treatment of acute CD: nitroheterocyclics belong both to BNZ – the first line of choice in the treatment – and nifurtimox (NFX), the second line of choice in the treatment of CD. NFX is used in cases of patients who are not suitable for treatment with BNZ, this inadequacy may be due to the adverse effects it triggers, or even due to the presence of infection by resistant strains (CASTRO; SOEIRO, 2017). In terms of mechanism of action, NFX acts similarly to BNZ. Reaching maximum plasma concentration 2 to 3 hours after its administration, it differs from BNZ because, instead of the formation of a nitro radical and direct binding to macromolecules, NFX undergoes reduction to a nitro-anion radical, which then binds to nucleic acids and inhibits the enzyme "Trypanothione reductase" (PUND; JOSHI, 2017). This enzyme is responsible for the protozoan's antioxidant defense and, when inhibited, leaves it exposed to harmful oxidant potentials that end up causing enough damage to culminate in its lysis (PUND; JOSHI, 2017).

A prospective cohort study developed in the United States, involving NFX, reaffirmed that the adverse effects induced by it, compared to BNZ, are less frequent. In that study, 53 chagasic patients were evaluated regarding the presentation of adverse effects during treatment with 8 to 10mg/kg of NFX, divided into three daily doses, for 12 weeks. All patients experienced adverse effects, mainly presenting nausea and anorexia, and of which 79% (42) completed treatment. The others stopped treatment due to symptoms such as depression, anxiety, drowsiness and rash. These symptoms, although less frequent, end up being more severe than those commonly observed using BNZ, which leads to more frequent but mild symptoms (FORSYTH *et al*., 2016).

Although NFX is generally used in acute phase treatments, it was pointed out that its use, even though it did not lead to a cure, caused a delay in the clinical progression of the chronic disease, as well as an improvement in the patients' prognosis due to the demonstration of a reduction in the parasite load in treated patients. Even so, the cost-effectiveness of treatment in the chronic phase must be evaluated, considering that many patients start to present more severe adverse effects when compared to patients in the acute phase, whether they are undergoing treatment with BNZ or with NFX (MENDES; SILVA; MARTINS, 2016). Furthermore, the Ministry of Health contraindicates NFX in patients who have both advanced chronic heart disease and early chronic heart disease, in addition to the chronic indeterminate or digestive form in adults. In these last three situations, BNZ is still indicated (CONITEC, 2018).

In addition to these nitroheterocyclics, another drug from the same family is being tested. This is Fexinidazole (FNZ), a drug approved in 2018 for the oral treatment of sleeping sickness, after demonstrating proven efficacy against *Trypanosoma brucei gambiense* (COSTA, 2018). The drug, rediscovered in 2005 by the DNDi (Drugs for Neglected Diseases Initiative), began to be tested in 2013 in patients with chronic-stage CD, and this study was suspended due to a failure in recruiting individuals. However, during the follow-up carried out in the 12 months before the suspension, there was efficacy in the use of FNZ. Currently, the study is being conducted again in Spain, with 45 chronic patients treated in a short regimen of 3, 7 and 10 days and whose follow-up had been taking place since September 2018, when the recruitment of the members of the sample had been completed. The follow-up phase of the trial was completed in 2019 and the results were expected to be released for 2021 (BARREIRA; BLUM, 2018; DNDi, 2019). Francisco and collaborators, indicated FNZ as a promising drug, using 30 mice, treated with BNZ, NFX, FNZ and FNZ sulfone (FNZs) in acute (14

days) and chronic (114 days) contamination. The result showed that the drugs FNZ and FNZ sulfone have a short-term curative capacity, being 5 days in acute phase mice, while the BNZ and NFX drugs need a longer treatment time, approximately 20 days. Despite this, they pointed out that BNZ and NFX are more effective in the early chronic phase (FRANCISCO *et al*., 2016).

A study responsible for evaluating FNZ in relation to adverse effects and tolerability in humans, seeking approval for its use in the treatment of African trypanosomiasis, did not show significant systemic toxicity. Daily doses ranging from 100mg/kg to 1000mg/kg/day were used. The assessment of cardiovascular, respiratory, neural, hepatic, plasma and reproductive parameters did not show significant adverse changes (TORREELE *et al*., 2010).

Aromatic diamidines: DB75, DB569, DB1362, MB17, MB18 and MB38

The class of aromatic diamidines (DAs) is composed of drugs that act against a wide variety of agents including bacteria, fungi and protozoa (DALIRY, 2011). Their mechanism of action has not been completely elucidated, however, one of the most studied trypanocide mechanisms is its ability to bind adenosine-rich sequences with thymine in DNA, leading to direct inhibition of replication and transcription of enzymes. (SOEIRO *et al*., 2005) It also binds to the kinetoplast, a mitochondrial region formed by concentrated extranuclear DNA, leading to its fragmentation and death of the parasite (MOREIRA; LÓPEZ-GARCÍA; VICKERMAN, 2004; SOEIRO *et al*., 2005; WILSON *et al*., 2005).

In vitro studies using furamidine (DB75) and its analogue DB569, with different forms and strains of *T. cruzi*, showed mitochondrial and nucleic ultrastructural alterations (SOUZA *et al*., 2004). A more detailed analysis showed type I programmed cell death (apoptosis) in a portion of the parasites. The results obtained by the analogue DB569 proved to be more active and with a higher number of cell deaths due to apoptosis (SOUZA *et al*., 2006a). When analyzed in vivo, the compound DB569 showed a decrease in the parasite load and fibrosis in the cardiac tissue, similar to the results obtained with the treatment with BNZ, in addition to resulting in a decrease in liver and kidney damage. The compound did not reduce the levels of parasitemia, however, the study indicates that this may be an influence of the affection of other tissues and/or its mechanism of action in reducing cardiac parasitemia was through synergism with inflammatory cells of the heart and/ or exacerbation of the microbicidal potential of these cells (SOUZA *et al*., 2006b).

There is also a study using the compound DB1362, whose results in the in vitro phase showed its active manifestation against the trypomastigote and amastigote forms of *T. cruzi*, presenting low toxicity to host cells. The use of this diamidine has been related to a profound change both in the organization of the kinetoplast and in the protozoan nucleus. The same study indicated, in experimental animals, a 40% reduction in parasitemia and 100% survival, using only two doses of 25mg/kg of DB1362 (SILVA *et al*., 2008).

The study by Girard *et al.* (2016) showed the analysis of the anti-*T.cruzi* effect of three new designated aromatic diamidines: MB17, MB19 and MB38. The three compounds showed a dose-dependent inhibitory effect on epimastigote growth. However, the compound MB17 showed remarkably better results, being the most potent inhibitor of both the proliferation of epimastigote forms and the motility of the parasites. Regarding its mechanism of action, MB38 causes loss of cytoplasmic membrane integrity, leading to a necrotic process in the *T. cruzi* cell in its epimastigote form, while the other two drugs do not induce damage to this structure. On the other hand, both MB17 and MB19 proved to be effective in inhibiting the growth of the parasite in its exponential phase by another mechanism, the former being more potent in this process and was studied further in the study.

Data about MB17 from the study by Girard *et al*. (2016) demonstrated that this drug binds to genomic DNA and to the kinetoplast, leading to DNA fragmentation, especially in the kinetoplast, and a significant decrease in ATP levels, which results in impairment of cell cycle processes and parasite replication. This compound was also effective in substantially decreasing the infectivity rate. Therefore, these data showed that even when dealing with three compounds within the same

pharmacological class, these drugs showed different results and efficiencies, with the MB17 compound being the most potent and promising for future studies on its anti-*T. cruzi* functions (GIRARD *et al*., 2016).

Scalene synthase inhibitors: WC-9 and its derivatives

Squalene synthase is responsible for catalyzing the reduction of farnesyl diphosphate molecules, which subsequently gives rise to a squalene molecule that is converted into various sterols. Squalene synthase inhibitors prevented the proliferation of intracellular amastigotes. By inhibiting this production, ergosterol synthesis is compromised, since squalene production is deficient, preventing the formation of a functional cell membrane in the trypanosome (ICHIKAWA *et al*., 2011).

Among the drugs in this class, Phenoxyphenoxyethylthiocyanate, known as WC-9, is the best-known squalene synthase inhibitor, being proven to be a potent agent against the proliferation of *T. cruzi* (LIÑARES *et al*., 2007). However, more recently, the incorporation of selenium in place of sulfur within the composition of the WC-9 formula brought about a dramatic increase in its effectiveness (WC-9) as *T. cruzi* proliferation inhibitors, making selenocyanate derivatives almost two-fold orders of magnitude more potent than thiocyanate counterparts with excellent selective index values (CHAO *et al*., 2019). Therefore, it can be said that selenium-containing derivatives proved to be extremely potent inhibitors of *T. cruzi* growth (CHAO *et al*., 2017).

CONCLUSION

It is concluded that there are promising studies to improve the treatment of CD. However, the development of effective drugs depends on numerous variables that must be circumvented aiming at a greater cost-benefit in the application of the drug, especially the reduction in the amount of side effects. Thus, drug efficacy is not the only variable that should be considered when balancing drug application. Many active ingredients that were shown to be effective in combating the pathogen ended up causing, in the long term, damage to the host's cells and were consequently discarded. Furthermore, the period of treatment and consequently the appearance of side effects, is a bias to be overcome, since BNZ itself, the drug of choice in the first-line treatment in Brazil to fight the disease (CASTRO; DIAS DE TORANZO, 1988) has a treatment dropout rate of two in three patients. Therefore, current research seeks to reduce the period of treatment while looking to increase success. Studies have shown great efficacy and low toxicity and have also been successfully administered in some countries in human trials. Despite this, these tests demand great financial capital for them to continue being developed, causing a hindrance that is difficult to overcome in Brazil and other endemic countries. Thus, the high complexity of *Trypanosoma cruzi*, added to these variables, slows down the development of new pharmacological strategies.

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