Current trends in the pharmacological management of Chagas disease

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Chagas disease (CD) is an endemic anthropozoonosis caused by the hemoflagellate protozoan Trypanosoma cruzi and transmitted mainly by hematophagous triatomines. CD is the most common fatal infection in Central and South America affecting, in endemic countries, about eight million people and causing the death of approximately 50,000 individuals each year (WHO, 2018). T. cruzi acute infection is usually asymptomatic and tissue damage may be detected decades after the first contact (Pérez-Molina and Molina, 2018). The main clinical alteration during the chronic stage are electrocardiographic abnormalities followed by contraction alterations that can lead to heart failure and, in many cases, to sudden death (Bocchi, 2017). CD cardiopathy results in progressive inability to continue working with a consequent overload of the country health system (Da Nóbrega et al., 2014).

No satisfactory treatment exists for CD, especially for the chronic stage of the disease (Morillo et al., 2015). The high costs associated with research and development of new drugs, coupled with the usually low financial return, results in the absence of new medicines. There is, consequently, an urgent need for novel alternatives and effective treatments for this disease. Several lines of research are presently being developed aiming to this objective, either trying to improve existing therapy or focusing in the development of new drugs. These topics will be reviewed in the present work that also intends to highlight the current perspectives on new approaches to the therapy of CD.

1. Available medicines for Chagas disease

After the first description of the disease, several compounds were tried as therapeutic agents (Fig. 1), such as arsenic, fuchsin, emetic tartrate and mercury chloride (Coura and Castro, 2002; Dias et al., 2009). However, all failed to produce satisfactory results. The antiseptic gentian violet was also used in the past, but it is currently used exclusively in blood banks as a prophylactic agent (Coura and Dias, 2009; Coura and Castro., 2002).

Since the 1970’s, several new compounds were introduced for the...
dazole Evaluation for Interrupting Trypanosomiasis
monstrated that the use of BNZ did not lead to clinical improvements in (Oliveira et al., 2017). Recently, the multicenter clinical trial abdominal swelling and some severe manifestations as eosinophilia number of side e adducts with guanosine bases in DNA and RNA (Kratz et al., 2018). Furthermore, BNZ may increase the phagocytosis and lysis of the release of dialdehyde glyoxal that has trypanocide e 2017). Also, BNZ could be reduced by a type I nitroreductase (NTR), refuting the oxidative stress as the determining factor (Hall et al., 2011; Boiani et al., 2010).

Because of its high toxicity, NF was progressively discontinued and its commercialization was suspended in Brazil, Argentina, Chile and Uruguay (Coura and Castro, 2002) from the 1980s. However, in these countries NF is retained as an option when treatment with BNZ fails, requiring authorization from PAHO or WHO for its use (Dias et al., 2016). Of note, resistance to nitroheterocyclic compounds have been reported (Mejia et al., 2012; Wilkinson et al., 2009), which seems to be associated with the loss of a single copy of the TcNTR gene (Wilkinson et al., 2008). Trying to solve toxicity and resistance limitations, clinical studies have been conducted to change the dose of NF tablet without losing effectiveness reviewed by Sales Junior et al., 2017.

Currently, the only drug available in most Latin American countries is benznidazole (BNZ). Initially produced by the pharmaceutical company Roche (Rochagan®), BNZ is now exclusively manufactured by the Pharmaceutical Laboratory of the State of Pernambuco (Lafepe), Brazil, and by the private laboratory Elea (Abarax®). BNZ is the N-benzyl-2-nitro-1-imidazoleacetcamide molecule. Different mechanisms of action have been attributed to BNZ. For example, it is suggested that it may act by a reductive stress, involving covalent modifications in DNA, proteins and lipids (Sales Junior et al., 2017). Also, BNZ could be reduced by a type I nitroreductase (NTR) present in the parasite, followed by several reactions that cause the release of dialdehyde glyoxal that has trypanocide effect by forming adducts with guanosine bases in DNA and RNA (Kratz et al., 2018). Furthermore, BNZ may increase the phagocytosis and lysis of the parasite and inhibit its growth by the action of the enzyme fumarate reductase-NADH (Dias et al., 2009; Sobrinho et al., 2007).

Low benefit in the chronic phase of the disease, regional variations in efficacy and emergence of resistant strains are some limitations of the clinical use of BNZ (Sobrinho et al., 2009). In addition, it causes a number of side effects such as rash, epigastric pain pruritus, nausea, abdominal swelling and some severe manifestations as eosinophilia (Oliveira et al., 2017). Recently, the multicenter clinical trial “Benznidazole Evaluation for Interrupting Trypanosomiasis” (BENEFIT) demonstrated that the use of BNZ did not lead to clinical improvements in patients with established Chagas’ cardiomyopathy when compared to the placebo group, even those with New York Heart Association (NYHA) class I or II heart failure, despite a reduction in parasite load (Morillo et al., 2015).

2. Repositioning of therapeutic drugs

Repositioning of established pharmacotherapeutic agents with well-known activity and side effect profiles is considered an effective strategy for the development of new treatments for several diseases, especially for neglected disorders. This repositioning approach is advantageous in view of the cost and time-consuming process required compared to the development of new medicines, since the drugs used in repositioning already have their toxicological and pharmacokinetic profile assessed when used on their previous therapeutic target (Alberca et al., 2016).

Several compounds of different pharmacological classes have already been tested against the T. cruzi (Table 1). Among them, benidipine, a calcium channel blocker usually administered for the treatment of hypertension and angina, and the antibiotic clofazimine were tentatively used against the parasite since they act inhibiting cruzipain, the main lysosomal cysteine protease with an important role in parasite infectivity. The inhibition of cruzipain has been much explored, because it is the most abundant protease of the parasite. Cruzipain has been shown to be crucial for all stage of the parasite life cycle and is involved in the parasite nutrition, invasion of mammalian cells and evasion of the host immune response (Dias et al., 2009; Rogers et al., 2012; Salas-Sarduy et al., 2017). Thus, benidipine and clofazimine were considered promising candidates for the treatment of the disease in view of their capacity to reduce the parasitic burden in the blood and skeletal tissues of infected mice, additionally curtailing the inflammatory effects of the infection. The use of these drugs in combination with current adopted therapy should be further evaluated (Sbaraglini et al., 2016).

Other repositioning drugs potentially useful in the ancillary treatment of Chagas disease were clomipramine, sertraline and fexinidazole. Clomipramine, a tricyclic antidepressant, was able to increase the survival of infected mice, reducing parasitemia, although it was not capable of eliminate the T. cruzi. Its beneficial action was initially associated to the inhibition of trypanothione reductase (TR), a unique enzyme that interacts with Flavin-adenine dinucleotide (FAD) and reduces nicotinamide adenine dinucleotide phosphate (NADPH), which is essential to parasite survival (Fauro et al., 2013; Benson et al., 1992). However, this hypothesis diverges from studies showing that the trypanocide effect would only be achieved by almost total inhibition of the TR, what is not obtained with this drug (Krieger et al., 2002; Mendonça et al., 2018). Sertraline is also indicated for the treatment of depression and anxiety, and its use in CD in vitro experiments showed efficacy against intracellular and bloodstream forms, with low cytotoxicity to
mammalian cells (Ferreira et al., 2018).

Fexinidazole is a promising broad spectrum antiparasitic agent with clinical trials already taking place. This compound has a nitro group in its composition that is metabolized by the parasite nitroreductases, forming reactive species that inhibits DNA synthesis (Bahia et al., 2012). Its composition that is metabolized by the parasite nitroreductases, early treatment can reduce heart inflammation associated with the chronic CD. It is well tolerated by the animals, without the occurrence of adverse effects. Of interest, fexinidazole has been approved by the European Medicines Agency (EMA) for the treatment of African Trypanosomiasis, in both adults and children (Deeks, 2019), thus demonstrating its therapeutic potential to be applied to CD. Probably, dose and treatment time adjustments may be required for clinical trials.

Dietary supplements have also been evaluated in CD treatment. Resveratrol, considered a food supplement by health surveillance agencies, has antioxidant and cardioprotective properties, acting in the chronic phase of the disease by non-trypansomidal mechanisms (Vilar-Pereira et al., 2016). Furthermore, resveratrol acts as a neuroprotector during CD, improving gliogenesis and neural migration (Fracasso et al., 2018).
Curcumin, a herbal supplement and a natural food coloring extracted from ginger, is a natural phenol that failed in reducing parasitemia, although lowering inflammatory leucocyte infiltration (Hernández et al., 2018).

Like curcumin, several repositioning attempts failed to achieve the expected results. For example, auronofin, a compound derived from gold, generally prescribed for the treatment of rheumatoid arthritis, showed ability to increase the survival of mice infected with the parasite without reducing parasitemia and with reduced selectivity (Da Silva et al., 2016). Nitazoxanide, a broad-spectrum antiparasitic drug that inhibits the parasitic intracellular enzyme pyruvate-ferredoxin oxidoreductase (PFOR) and blocks the ion channel of glutamate chloride, disclosed poor results against the T. cruzi worsening the infection in mice, increasing tissue damage in relation to untreated animals, and exhibiting an increased mortality. Consequently, this compound was considered highly unsafe (Valle-Reyes et al., 2017).

Other drugs comprised in the strategy of therapeutic repositioning belong to the class of sterol biosynthesis inhibitors that are components of the parasitic membranes and essential for the cellular growth (Dias et al., 2009). A 20-year follow-up study of patients treated during 120 days with itraconazole, a synthetic analogue derived from imidazole, showed the drug capacity to interrupt or delay the natural course of CD during the chronic phase, causing regression of some abnormalities on the electrocardiogram and showing few adverse effects (Apt et al., 2013). However, in this research, problems in the control group and poor sensitivity of the used tests to determine cure were observed; so further studies are needed to confirm the findings.

The prospective, randomized clinical trial of CHAGASAZOL evaluated the efficacy and safety of posaconazole, an antifungal that actively inhibits the synthesis of ergosterol, the main sterol of the parasite. Contrary to previous findings in vitro and in experimental models of Chagas disease, which posaconazole showed excellent activity, the drug failed to disclose the same expected efficacy in humans (Molina et al., 2014). The results in the human trial were recapitulated in a study using bioluminescent parasites in a mouse model, which confirmed the low efficacy of posaconazole in both acute and chronic phases of the disease (Francisco et al., 2015). The use of ravuconazole was evaluated in a phase 2 clinical trial in patients in undetermined chronic phase, without cardiac damage. Of interest, it was observed that the prodrug E1224 led to parasite clearance, but with transient response when using low- or short doses regimens (Torrico et al., 2018).

3. Drug association

The concomitant or sequential use of two or more pharmaceutical compounds represents an alternative that is also being explored in CD treatment studies (Table 2). Theoretically, the combination of different compounds allows the reduction of the doses and/or treatment length, resulting in reduction of drug adverse side effects and costs. Synergistic treatment generally improves compounds activity with distinct mechanisms of action, and additionally reduces the drug toxicity and the chance of developing resistance (Ferreira and Andricopulo, 2016; Andrews et al., 2014).

A successful association between itraconazole and BNZ was demonstrated in a murine model. The concomitant administration of both drugs allowed a decrease in BNZ dose by 25% and on time needed to curtail the parasitemia. In addition, mice treated with this drug combination showed lessened lesions in the cardiac tissue and fewer inflammatory cells associated with the chronic phase of the disease (Martins et al., 2015). Itraconazole seems to alter the pharmacokinetic profile of BNZ, increasing its half-life and its volume distribution extent, allowing an increased accumulation of BNZ in the biological systems (Da Silva et al., 2012; Martins et al., 2015).

Diniz et al. (2013), using a seven-day rapid treatment protocol, observed that the combination of BNZ and posaconazole was more effective compared to using each one alone. Additionally, the association allowed reduced doses compared to individual administration.

The combination of BNZ and clomipramine showed a synergic activity against T. cruzi (Strauss et al., 2018), and attenuated necrosis and liver damage, possibly due to the hepato-protective effect of the antidepressant. Effectively, mice treated with the association of these drugs disclosed less renal damage when compared to the group treated with BNZ alone (Strauss et al., 2013). Besides that, they showed reduced parasitemia and mortality rate (Strauss et al., 2018).

In a murine model of chronic Chagas cardiomyopathy the concomitant use of simvastatin, a lipid-lowering medication, and BNZ decreased fibrosis and inflammation. Importantly, both drugs are well-studied, with a well-established safety profile, requiring no further expensive and long-term clinical studies (González-Herrera et al., 2017). Furthermore, the sequential use of BNZ and allopurinol (used in the treatment of gout) was well tolerated in humans, leading to beneficial therapeutic immunological changes during the chronic CD, significant reduction of parasitic loads, absence of electrical abnormalities in the heart, and increased cure rate (Perez-Mazilah et al., 2012; Rial et al., 2018; Mazzetti et al., 2019). Other combinations with BNZ that have shown to be interesting and deserve better investigation are ascorbic acid (Providello et al., 2018), hydroxymethylfurfuralzone (Scaram et al., 2018a,b), and imidazole (De Araújo et al., 2019).

4. New drugs

Despite the high cost and the time required to search new compounds, this is the main strategy to identify novel treatments for CD in recent years (Table 3). These new drugs, of natural or synthetic origin, are being tested but they still in the pre-clinical phase. Most of them show activity against the parasite, however, in many cases, their mechanisms of action have not yet been elucidated. For example, studies with Cordycepin/Pentostatin and with the Piper jericocoense plant showed that these compounds have trypanocidal effects, but it is not completely known in which pathways of the parasite they are acting (do Carmo et al., 2019; García-Huerta et al., 2018).

Psilostachin (Ps) and Psilostachin C (PsC) (sesquiterpene lactones) are natural compounds found in the Asteraceae family that have displayed activity against the parasite by different mechanisms of action. Psi possibly interacts with the heme group, and PsC inhibits the synthesis of ergosterol. Both compounds showed interaction with hemin, a product of hemoglobin digestion essential for parasite survival (Sülsen et al., 2016). Of interest, the sesquiterpene lactone isolated from Lycophthora trichocarpa led with nanoparticles was able to reduce parasitemia and increases the lifetime of treated animals (Branquinho et al., 2014).

Natural chemical constituents isolated from the cashew nut Anacardium occidentale showed trypanocidal effects similar to those of benznidazole, by inhibition of T. cruzi sirtuins (Bastos et al., 2019). Furofuran lignan and Eupomatenoid-5, isolated from the leaves of Piper jericocoense and Piper regnellii respectively, acted against the three evolutionary forms of the parasite (Garcia-Huerta et al., 2018; Lazarin-Bidóia et al., 2013). The last decreases the activity of the enzyme trypanothione reductase, resulting in increased amount of oxygen and nitrogen reactive species and the death of T. cruzi. Interestingly, thiazolidine LPSF SF29, a synthetic compound, interferes with the biosynthesis of the substrate of the enzyme trypanothione reductase, also causing mitochondrial dysfunction and parasite death (De B. Moreira et al., 2013).

Several molecules with probable mechanism of action on the parasite sterol biosynthesis were also tested. The enzyme sterol 14-Demethylase (CYP51) is essential for the synthesis of sterols, and its inhibition is used as a pharmacological target. Azole sterol biosynthesis inhibitors, such as, VNI (R)-N-(1-(2,4-dichlorophenyl)-2-(1H-imidazole-1-yl)ethyl)-4-(5-phenyl-1,3,4-oxadiazol-2-yl)benzamide, inhibitor of protozoan CYP51, and VNF, (R)-4-chloro-N-(1-(2,4-dichlorophenyl)-2-(1H-imidazole-1-yl)ethyl)benzamide, increased the
| References                              | Drug association/original indication                  | Culture type/animal type                      | Main Results                                                                                                                                                                                                 |
|----------------------------------------|------------------------------------------------------|-----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Da Silva et al., (2012)                | Itraconazole (Antifungal) and Benznidazole (Antiprotozoal) | Swiss albino mice, Patients (human)           | Itraconazole alters the pharmacokinetic profile of benznidazole with accumulation in vivo, which represents a benefit as it provides the necessary dose reduction. The sequential treatment with the drugs was well tolerated by the patients and considered viable. Immunological changes were detected giving benefits to patients with a disease in the chronic phase. |
| Perez-Mazliash et al., (2012)          | Allopurinol (Antigout) and Benznidazole (Antiprotozoal) | Patients (human)                              | The association with C4 provided a reduction in the dose of benznidazole, achieving the same results as the usual dose. The association of benznidazole with other drugs, in short periods, can cause the elimination of the parasite, except the association with amphotericin B. The association with nifurtimox led to the behavioral alteration of treated animals. |
| Valdez et al., 2012                    | C4 (new drug) and Benznidazole (Antiprotozoal)         | LLCMK2 and Balb/c mice                        | The compounds interfered in the growth of the epimastigote form, in a dose-dependent manner. In all evolutionary forms, there are structural alterations in the parasite, with the formation of structures that indicate autophagy. They presented synergism when administered together, being able to reduce the parasitism to values inferior to those obtained with administration of the drugs alone. |
| Cencig et al., 2012                    | Benznidazole and Nifurtimox, Posaconazole or Amphotericin B (Antiprotozoal; Antifungal) | Balb/c mice                                   | Prevented cardiac conduction abnormalities in animals. In addition, the association prevented the formation of perivascular necrosis and inflammation in the liver. |
| Veiga-Santos et al., 2012              | Amiodarone (Antiarrhythmic) and Posaconazole (Antifungal) | ND*                                          | The association of drugs led to less need for days of treatment, and reduced tissue damage caused by the disease in treated animals. |
| Diniz et al. (2013)                    | Benznidazole (Antiprotozoal) and Posaconazole (Antifungal) | Swiss mice                                    | In the murine model the association increases BNZ activity. Since 5-epi-lipoxin A4 induced by simvastatin treatment could improve the pathophysiological condition of patients. |
| Strauss et al. (2013)                  | Clomipramine (Antidepressant,Tricyclic) and Benznidazole (Antiprotozoal) | Swiss mice                                    | In vivo were analyzed, showing increased BNZ effect, survival, trypomastigote decrease and lower levels of anti-T-cruzi Ig G 34 dpi. |
| References                  | New drugs (compound)                      | Culture type/animal type | Main Results                                                                                                                                                                                                 |
|-----------------------------|-------------------------------------------|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ramírez-Macias et al., 2012| Terpenoid derivatives                     | Vero cells and Balb/c mice | The trypanocidal activity of the derivatives was slightly higher (1 and 2) with respect to that found for BZN. Reduced the growth capacity of the parasite, its multiplication and differentiation. Addition in order to induce mitochondrial changes in the epimastigote. |
| Becco et al., 2012          | Casiopelas® (copper complexes)            | T. cruzi (Dm28c strain) against proliferative esukaryotic cells LLC-MK2 cells | The compounds tested showed similar results to nifurtimox.                                                                                                                                                |
| Da Silva et al. (2012)      | Arylimidamide and its mesylated salt form  | T. cruzi (Dm28c strain) against proliferative esukaryotic cells | Trypanocidal effect against both relevant forms in mammalian hosts in vivo: The compounds presented significant selectivity, DB1965 shows high activity in acute experimental models. They reduce parasitemia, and decrease animal mortality. |
| Santos et al., 2012         | Eugenia jambolana (natural extract)       | Murine J774 macrophages  | The compound exhibits activity against the relevant epimastigote form, with reduced toxicity.                                                                                                               |
| Polanco-Hernández et al., 2012 | R. paluchra, S. yucatanenensis, S. villosa, and B. baculifolia | Vero cells and Balb/c mice | The leaf extracts of S. yucatanenensis and B. paluchra were the most active against and trypanomastigotes. Only the S. yucatanenensis extract showed significant trypanocidal activity in vivo, reducing 75% of parasitemia. |
| Higa et al., 2013           | Archaea (to act as adjuvant for soluble parasite antigens) | LLC-MK2 cells | Immunization of the animals with the vaccine was able to limit the course of infection in terms of parasitemia and mortality. induced ultrastructural changes, such as flagellar detachment, intense mitochondrial edema, formation of myelin-like figures and appearance of autophagosomes. |
| Moreira et al., 2013        | Thionoilde LPSF SF29 (organic compound)   | LLC-MK2 cells | The compounds possess activity against the three forms of the parasite, involving alterations induced by oxidative stress.                                                                                     |
| Gaziantep et al. (2013)     | Eupomatiosid-5 (isolated from P. reneglesi) | LLCMK2 cells | The compound presents activity in the acute and chronic phase, with reduction of parasitemia in the animals.                                                                                               |
| Villalta et al. (2013)      | VNI (Experimental inhibitor of T. cruzi 14α-demethylase) | Cardiomyocyte tissue culture and Balb/c mice | Dose-dependent trypanocidal activities against bloodstream trypanomastigotes. The compounds have effective activity against the parasite, with trypanocidal potential, low cytotoxicity and reduced parasitemia. |
| Soeiro et al. (2013)        | VNI and its derivative VNF                | Mammalian cell cultures and Swiss mice | The analogs were reduced in toxicity as a mutagenesis of the analogs was detected at a concentration greater than the lower concentration of megazole. Among the compounds tested, some showed the ability to reduce parasitemia, two compounds did not present in vivo activity. |
| Mello et al., 2013          | Nitroimidazole, thiazidazole, megazole (Nitro analogs) | RAW264.7 macrophages | The compounds tested showed similar results to nifurtimox. The compounds showed activity against the parasite with a dose-dependent reduction of parasitemia. |
| Papadopoulos et al., 2013   | Novel 3-nitro-1H-1,2,4-triazole (Based compounds) | Caco-2 cells, L6 cells and Balb/c mice | The compounds showed activity against the parasite, involving alterations induced by oxidative stress.                                                                                               |
| Jiménez-Coello et al., 2013 | Carica papaya (Seed extract)              | Balb/c mice | The compounds showed activity against the parasite, involving alterations induced by oxidative stress.                                                                                               |
| Raviolo et al., 2013        | 6N-alkyl (2a-c) and N1-acyl (3a-c)        | Mammalian cells (murine splenocytes) and Vero cells | Only 1 of the compounds, called 3c, has activity against the parasite, with better solubility and lipophilic than allopurinol, in addition to a reduction in cytotoxicity. |
| Hargrove et al. (2013)      | UDO e UDD (pyridine derivative)           | L6 cells and Swiss mice | The compounds are effective against T. cruzi, being selective against the parasite. The heme-heterocycle and apoprotein-ligand interactions may be helpful in minimizing the off-target activity of CYP51 inhibitors and directing the design selective drugs. |
| Fonseca-Berzal et al., 2013 | Tetrabordroquinolines (Organic compound, derivative of quinoline) | Murine fibroblasts and Vero cells | The compounds showed in vivo activity against the parasite, without cytotoxicity. The compounds tested showed in vivo activity against the parasite, without cytotoxicity. |
| Adade et al., 2013          | Melittin (antimicrobial peptides)         | LLC-MK2 cells | The compounds showed in vivo activity against the parasite, without cytotoxicity. The compounds tested showed in vivo activity against the parasite, without cytotoxicity. |
| Esperandi et al., 2013      | CUR (isolated from P. cubeba) and HNK (obtained from CUR) | Balb/c mice | The compounds showed in vivo activity against the parasite, without cytotoxicity. The compounds tested showed in vivo activity against the parasite, without cytotoxicity. |
| Branquinho et al. (2014)    | Lycnopholide Sesquiterpene lactone, isolated from Lycnophora trichocarpha | Swiss mice | The compounds showed in vivo activity against the parasite, without cytotoxicity. The compounds tested showed in vivo activity against the parasite, without cytotoxicity. |
| Cazorla et al., 2014        | Attenuated Salmonella.                    | Vero cells and CH1/HeN | The compounds inhibited the growth of the amastigote forms of the parasite and caused lysis of the trypanomastigote form, presenting low cytotoxicity against the host. |
| Adade et al., 2014          | Crovirin (Crude venom extract)            | LLC-MK2 cells | The compounds inhibited the growth of the amastigote forms of the parasite and caused lysis of the trypanomastigote form, presenting low cytotoxicity against the host. |
| Caballero et al., 2014      | Triazolopirimidine compounds (six newly synthesized transition metal complexes) | J774.2 macrophages and Balb/c mice | The compounds showed in vivo activity against the parasite, without cytotoxicity. The compounds showed in vivo activity against the parasite, without cytotoxicity. |
| Matos et al., 2014          | Trc52 amino-terminal-domain DNA (Carried by Salmonella enterica) | COS-7 cells, Vero cells, Spleen cells and CH1/HeN mice | The compounds showed in vivo activity against the parasite, without cytotoxicity. The compounds showed in vivo activity against the parasite, without cytotoxicity. |
| Varela et al., 2014         | Aristeguietia glutinosa (Hydro-Ethanolic Extract and Isolated Active Principles) | Balb/c mice | The compounds showed in vivo activity against the parasite, without cytotoxicity. The compounds showed in vivo activity against the parasite, without cytotoxicity. |
| Carneiro et al., 2014       | H. J bdte (Dithiocarbamate complexes)     | Spleen cells isolated from CS7BL/6 mice and Swiss mice | The compounds showed in vivo activity against the parasite, without cytotoxicity. The compounds showed in vivo activity against the parasite, without cytotoxicity. |

(continued on next page)
Table 3 (continued)

| References | New drugs (compound) | Culture type/animal type | Main Results |
|------------|----------------------|--------------------------|--------------|
| Moraes et al., 2014 | Nitroheterocyclic compounds and four ergosterol biosynthesis inhibitors | U2OS cells and LLC-MK2 | The heterocyclic nitro compounds presented superior efficacy to the other compounds tested. Oxaborad AN4169 is a candidate with potential for broad-spectrum activity and for favorable trypanocidal kinetics. |
| Veiga-Santos et al., 2014 | KH-TFMDI (sirtuin inhibitor) | LLC-MK2 cells | It presented an inhibitory effect against the three evolutionary forms of the parasite and induced lysis of the trypanostatic form. It caused alterations in the structure of the parasite, such as loss of stability and autophagy, besides having high selectivity. |
| Olmo et al., 2014 | 5 derived compounds N, N squaramides (amide-type compounds) | Vero cells and Balb/c mice | Four showed greater in vitro activity than benznidazole, reducing the number of amastigotes in infected cells. In vivo, the compound succeeded in eradicating the parasites in most animals. |
| Papadopoulo et al. (2015) | Nitrotiazole-based derivative | L6 cells and Balb/c mice | The 3-nitrotiazole compounds showed potent and selective anti-T. cruzi activity. |
| Cortes et al., 2015 | Four gallic acid derivatives | Vero cells | The compounds showed high selectivity, and two were more potent against the parasite superior to nitrofurazone. |
| Meira et al., 2015 | Physalis oasae L. (Ethanolic extract) | LLC-MK2 cells and Balb/c mice | The extract inhibited the proliferation of the epimastigote form of the parasite and induced lysis of the trypanostatic form. In vivo analysis, it was able to reduce parasitemia. |
| Montesino et al., 2015 | 58 extracts of medicinal plants | L6 cells | Of the extracts tested only Valeriana officinalis presented activity against T. cruzi, Salvia, Valeriana, Hypericum, Silybum, Arnica, and Curcuma exhibited high activity. |
| Suto et al., 2015 | Komaroviquinona (quinones derived) | Swiss3T3 cells and HT1080 cells | Four of the synthesized compounds showed considerable effect against T. cruzi and had low cytotoxicity. Further studies are in progress. |
| Papadopoulo et al. (2015) | 3-nitrotiazole (Based amides and carbonols) | L6 cells, Caco-2 cells and Balb/c mice | Reduced the parasitemia in the acute experimental model of the disease. The bifunctional compounds were able to completely clear the parasites after 10-day treatment |
| Santos et al., 2015 | Porecia macrocarca (acetylene fatty acid derivatives) | NCTC cells | The derivative 1 exhibited activity against trypanostatic forms of T. cruzi ten times more effective than the benznidazole. |
| Palas-Belel et al., 2015 | 5- substituted hydrazides (compounds derived) | LL-24 human fibroblast cells | All of the derivatives, except for one, showed increased trypanocidal activity against the three strains compared to RZD. 62% of the compounds were more active than nitrofurazone against the Y strain. |
| Mendoza-Martinez et al., 2015 | Quinazoline derivatives | Vero cells and CD1 mice | 4 of the 9 compounds (44%) presented higher activity than the reference drugs. |
| Wong-Bazea et al., 2015 | Benzyl ester of N-propyl oxamate | Vero cell line and NIH albino mice | The polar NPOx showed no trypanocidal activity. In contrast, the hydrophobic B-NPOx ester exhibited trypanocidal activity in vitro and in vivo. |
| Alvarez et al., 2015 | Amide-containing thiazole | Vero cells and CD-1 mice | Thiazole 4 was active against trypanostatic forms and prevented the intracellular growth of amastigotes. Thiazole 4 suppressed parasitemia by modifying the anti-antibodies as the reference drug. |
| Olmo et al., 2015 | Abietic acid derivatives (diterpenoid) | Vero cells and Balb/c mice | The three compounds were shown to be better inhibitors of trypanoithione reductase than Nifurtimox. |
| Neitz et al., 2015 | GNF7198 (xanthine analogue) | C2C12 cells, 3T3 cells, Caco-2 cells and Balb/c mice | The xanthine analogs showed in vitro activity against the parasite and were able to reduce parasitemia more than benznidazole in the animal model. |
| Khare et al. (2016) | GNF 6702 (selective inhibitor of the kinetoplastid pyrrolase) | NIH 3T3 fibroblast cells and Balb/c mice | The compound showed activity in the parasitemia reduction, confirmed after immunosuppression of the animals. |
| Sulsen et al. (2016) | Psilostachyin and Psilostachyin C (Sesquiterpene Lactones) | Action of 2 compounds on T. cruzi | The compounds possess activity against the parasite by different mechanisms of action. The association these compound may be further investigated as a new therapeutic modality for the treatment. |
| Arias et al., 2016 | Nitrofuram derivatives | HeLa cells and mammalian cells | The three compounds were shown to be better inhibitors of trypanoithione reductase than Nifurtimox. |
| Fonseca-Berza et al., 2016 | 5-nitroindazole derivatives | Mammalian cells (fibroblasts), Vero cells and cardiac cells | Many series A indazoliones were efficient against different morphological forms of the CL Brener strain of T. cruzi. |
| Farrow et al., 2016 | Adenovirus 48 (Ad48) | HEK293 cells and C57BL/6 mice | Mice that were immunized with the modiﬁed vectors were able to induce speciﬁc humoral and cellular responses of T. cruzi. Only two compounds named 3 and 5 extracted from plants Dorstenia mannii, and Pentas schimperi respectively, showed activity against T. cruzi in a moderate manner. |
| Sandjo et al., 2016 | Six plant meta boites | THP-1 cells | The compound increased the survival of the treated animals, also decreasing parasitemia. |
| Lozano et al., 2016 | Diterpene 5-epi-icetexone (Salvia gilliesii.) | Swiss albino mice | Therapeutic vaccines were able to modify the Th1 proﬁle response by protecting and sustaining not only C2 but also against a variety of parasite antigens. |
| Cerny et al., 2016 | Cruzipain and GM-CSF DNAs, | C3H/HeN mice | The amide of dehydroabietylamine compound was highly effective against amastigote forms of T. cruzi resident in L6 cells in addition to high selectivity, superior to benznidazole. |
| Pirtitama et al., 2016 | Abietane diterpenoid | L6 cells | Drastic reduction of parasitemia in the two phases of infection. In addition to preventing damage to cardiac tissue caused by inflammation. |
| Calvet. Et al., 2017 | 4-aminopyridyl-based (CYP51 inhibitors) | Swiss mice | VPF caused a > 99.7% reduction in peak parasitemia, while NIV values ranged from 91 to 100%. |

(continued on next page)
Table 3 (continued)

| References                          | New drugs (compound)                  | Culture type/animal type | Main Results                                                                                                                                 |
|-------------------------------------|---------------------------------------|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Brand et al., 2017                  | 5-amino-1,2,3-triazole-4-carboxamides  | Vero and HepG2 cells NMRI mouse and Balb/c mice | The optimization of the ATC series favored solubility and metabolic stability in oral administration. Study demonstrated activity against trypanomastigotes in the bloodstream, metacyclic trypanomastigotes and against epimastigotes and intracellular amastigotes, with a decrease in infection rate and low toxicity. The phase was found antiparasitic effect. Presented low toxicity, DB1957 allowed 100% animal survival and DB1959 and DB1890B did not reduce the circulating parasitism. Trypanocidal effect on amastigote and trypanomastigote. An interesting candidate for use in the treatment of potentially contaminated RBCs bags at low temperature. Deoxyxymikanolide presented the highest selectivity index for trypanomastigotes and amastigotes. Able to decrease parasitemia and weight loss associated with the acute phase. Permeabilization of the plasma membrane, as well as in the depolarization of mitochondrial membrane potential, leading to the death of the parasite. Prevent cardiac changes induced in vivo with minimal cardiotoxicity. The ABQs mediate their activities, with features that facilitate the inhibitory effects of antiparasitic growth, could be incorporated into novel and safer compounds. Modify the infection-induced immune response, with attenuated parasitism, and improve protection against new infections. 5 and 11 were as potent against T. cruzi as against the recombinant form of cruzipaine, which is generally considered a valid target. It reduced parasitic loads from 76% to 99%, compared to a variety of different controls, in addition to significantly reducing cardiac changes. |
| Cupello et al., 2017                 | LQ8 123 (PhenyI-t-ButylIntrone Derivative) | Mammalian Cells |                                                                                                                                               |
| Da Silva et al., 2017               | DB1957, DB1959 and DB1890 (Arylimidamides) | Caeco-2 cells and Swiss Webster mice |                                                                                                                                               |
| Papadopoulou et al., 2017           | Nitrotriazole-Based Compounds          | Swiss mice             |                                                                                                                                               |
| Villamariz et Al., 2017             | Linoolod (Piper aduncum essential oil)  | Vero cells              |                                                                                                                                               |
| Laurenla et al., 2017               | sesquiterpene lactones (isolated from Mikania plants species) | RAW 264.7 and Balb/c mice |                                                                                                                                               |
| Alexandre et al., 2017              | Ergosterol (basidiomycte Fleurus salmoneastramineus) | Mammalian cells         |                                                                                                                                               |
| Branquinho et al., 2017             | Lycnoholephile (natural substance)     | CSBL/6 mice             |                                                                                                                                               |
| Meredith et al., 2017               | Aziridinyl 1,4-benzoquinone (ABQ) (Quinone-based compounds) | THP-1 cells             |                                                                                                                                               |
| Brandão et al., 2017                | Live attenuated parasites in combination with plasmid pXVR-mIFN-γ | CSBL/6J mice            |                                                                                                                                               |
| Burtoloso et al., 2017              | Non-peptidic nitrile-based cysteine protease inhibitors | LLC-MK2 cells Balb/C 3T3 clone |                                                                                                                                               |
| Kunduri et al. (2017)               | Dendritic cells transduced with the adjuvant | A31 cells               |                                                                                                                                               |
| Sposito et al., 2017                | Rauvoucanazole (triazole antifungal)   | H9c2 cells and Swiss mice | Results obtained demonstrated a marked improvement in rauvoucanazole anti-T. cruzi activity when associated with SEDDS. The best performance was obtained with PLA-PEG NC, PCL NC also showed promising results. In addition to improving LYC pharmacokinetic parameters (iv), the NC protected the encapsulated LYC from degradation in mouse plasma. Active against all parasite forms and presented lower toxicity than Benznidazole. Reduced parasitism in acute phase Results confirm the ability of both TSA-1 and Tc24 recombinant proteins to recall an immune response induced by native antigens during natural infection. It reduced parasitic loads from 76% to 99%, compared to a variety of different controls, in addition to significantly reducing cardiac changes. Higher anti-Trypansoma cruzi activity than benznidazole.Reduction of parasitism during acute phase. |
| Branquinho et al., 2017              | Lycnoholephile (ipopolific sesquiterpene lactone) | ND*                     | Results obtained demonstrated a markedly improved in rauvoucanazole and anti-T. cruzi activity when associated with SEDDS. The best performance was obtained with PLA-PEG NC, PCL NC also showed promising results. In addition to improving LYC pharmacokinetic parameters (iv), the NC protected the encapsulated LYC from degradation in mouse plasma. Active against all parasite forms and presented lower toxicity than Benznidazole. Reduced parasitism in acute phase Results confirm the ability of both TSA-1 and Tc24 recombinant proteins to recall an immune response induced by native antigens during natural infection. It reduced parasitic loads from 76% to 99%, compared to a variety of different controls, in addition to significantly reducing cardiac changes. Higher anti-Trypansoma cruzi activity than benznidazole.Reduction of parasitism during acute phase. |
| García-Hueratz et al. (2018)        | Furofurian lignan (Piper jericose)     | Vero cells and Balb/c mice |                                                                                                                                               |
| Villanueva-Lizama et al. (2018)     | TSA-1 and Tc24 antigens               | Patients (human)        |                                                                                                                                               |
| Paixão et al. (2019)                | 1,10-phenanthroline                  | L929 cells and Balb/c mice |                                                                                                                                               |
| Miana et al. (2019)                 | 2,2-bipyridine                       | Vero cells              | Bipotency against epimastigotes and amastigotes forms, with less cytotoxicity than Benznidazole. Trypanocidal efficacy in acute and chronic Chagas disease, with lower toxicity than benznidazole. Trypanocidal activity, Eupoxystafitatin was the most active compound against T. cruzi trypanomastigotes and amastigotes Inhibition of the enzyme sirtuin, with anti-T. cruzi effects similar to those of benznidazole. Effective against all T. cruzi morphological forms, displaying lower cytotoxicity than Benznidazole. |
| Martín-Escolano et al. (2019)       | N-arylsulfonyl benzimidazoles         | Vero cells              | Bipotency against epimastigotes and amastigotes forms, with less cytotoxicity than Benznidazole. Trypanocidal efficacy in acute and chronic Chagas disease, with lower toxicity than benznidazole. Trypanocidal activity, Eupoxystafitatin was the most active compound against T. cruzi trypanomastigotes and amastigotes Inhibition of the enzyme sirtuin, with anti-T. cruzi effects similar to those of benznidazole. Effective against all T. cruzi morphological forms, displaying lower cytotoxicity than Benznidazole. |
| Sülen et al. (2019)                 | Estafetin (Sesquiterpene lactones derivatives) | Vero cells              | Trypanocidal activity, Eupoxystafitatin was the most active compound against T. cruzi trypanomastigotes and amastigotes Inhibition of the enzyme sirtuin, with anti-T. cruzi effects similar to those of benznidazole. Effective against all T. cruzi morphological forms, displaying lower cytotoxicity than Benznidazole. |
| Bastos et al. (2019)                | Natural compounds isolated from casheen nut (Anacardium occidentale, L. Anacardiaceae) | hFB cells              | Trypanocidal activity, Eupoxystafitatin was the most active compound against T. cruzi trypanomastigotes and amastigotes Inhibition of the enzyme sirtuin, with anti-T. cruzi effects similar to those of benznidazole. Effective against all T. cruzi morphological forms, displaying lower cytotoxicity than Benznidazole. |
| Martín-Escolano et al. (2019)       | AS-48 (Bacteriocin)                   | Vero cells              | Trypanocidal activity, Eupoxystafitatin was the most active compound against T. cruzi trypanomastigotes and amastigotes Inhibition of the enzyme sirtuin, with anti-T. cruzi effects similar to those of benznidazole. Effective against all T. cruzi morphological forms, displaying lower cytotoxicity than Benznidazole. |
| Do Carmo et al. (2019)              | Cordycepin                           | HeLa cells and swiss mice | Potent trypanocidal effect in vivo. However, reduced curative effect in vivo. Trypanocidal activity, Eupoxystafitatin was the most active compound against T. cruzi trypanomastigotes and amastigotes Inhibition of the enzyme sirtuin, with anti-T. cruzi effects similar to those of benznidazole. Effective against all T. cruzi morphological forms, displaying lower cytotoxicity than Benznidazole. |

ND* not determined.
Still regarding synthetic drugs under investigation, the compound GNF6702, proposed by Khare et al. (2016), showed significant activity against _T. cruzi_ by inhibiting the proteasome chymotrypsin-like activity, without any action on human proteasome. Currently, this compound is undergoing pre-clinical toxicological tests.

5. Conclusion

Although CD was discovered more than 100 years ago, the treatment remains inefficient for patients already in the heart phase of the disease. Several strategies have been followed to improve therapeutic treatment against CD: the use of the approved drugs BNZ and NF, the repositioning drugs from other pharmacological classes, the association of different compounds and through the search for more effective new drugs.

From this review, it is possible to conclude that: (a) despite the limited activity of BNZ in the chronic CD, nowadays this is the only treatment available in developing countries, such as Brazil. Thus, is imperative to develop new alternatives drugs in view of the detrimental treatment available in developing countries, such as Brazil. Thus, it is imperative to develop new alternatives drugs in view of the detrimental effects of the infection during the symptomatic chronic phase; (b) the use of BNZ in combination with other compounds has shown advantages compared to its use alone; (c) the search for new pharmacological alternatives has been the most used strategy to find new effective drugs; (d) most of the current studies are either at the stage of animal experimentation or in preclinical phases; (e) the inhibition of the parasite ergosterol biosynthesis has been widely explored as a pharmacological target, especially with the use of antifungal drugs, although poor clinical results.

6. Brief perspective

An innovative line of research, which seeks to use unconventional strategies can be currently observed. For example, the study by Villanueva-Lizama et al. (2018) suggests the possibility of employing vaccines as a potential pharmacological treatment. Using the antigens trypomastigote surface antigen-1 (TSA-1) and flagellar calcium binding protein of 24 kDa (Tc24), researchers showed that corresponding recombinant proteins have the ability to stimulate the immune system, establishing an immune response against these antigens during the infection natural course. In turn, Conduri et al. (2017) produced a vaccine based on dendritic cells, transduced with the dmSH adenoviral construct and loaded with the Tc24 recombinant protein to reduce or prevent cardiac complications of CD.

In addition, many studies utilize computational approaches to identify potential inhibitors of _T. cruzi_ crucial molecular targets (Scarmi, et al., 2018a,b). Melo-Filho et al., 2019 used a virtual screening based on quantitative structure-activity relationships (QSAR) model to prospect novel molecules against the _T. cruzi_ in commercial database. This model made possible the identification of seven potent and selective compounds against the parasite, which were validated in in vitro experiments. In another study, the virtual screening approach was used on a protein-based pharmacophore to detect potential targets of the glycolytic pathway of the parasite, enabling the identification of three distinct inhibitors of the glyceraldehyde-3-phosphate dehydrogenase from _T. cruzi_ (Maluf et al., 2013).

**Declaration of competing interest**

None.

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