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NEW INSIGHTS IN CHAGAS' DISEASE TREATMENT

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SUMMARY

Chagas' disease, caused by the kinetoplastid protozoon Trypanosoma cruzi, remains the highest parasitic disease burden in the American continent and is now spreading to nonendemic countries due to international migrations. Specific therapy for this complex condition remains unsatisfactory due to limited efficacy and common side effects of currently available drugs (nifurtimox and benznidazole), as well as controversies regarding the pathogenesis of the disease in the chronic stage. In contrast to long-held views on the autoimmune origin of the pathological manifestations of the chronic stage of the disease, recent studies have concluded that the persistence of parasites is the key factor leading to the sustained inflammatory responses underlying the characteristic lesions of chronic Chagas' disease, and that this condition should be treated as an infectious and not an autoimmune disease. Among the most promising approaches to new treatments are ergosterol biosynthesis inhibitors, such as posaconazole and ravuconazole, which are poised to enter clinical trials in Chagas' disease in the short term; the antiarrhythmic drug amiodarone, which was recently shown to also have potent activity against T. cruzi; inhibitors of cruzipain, the main cysteine protease of T. cruzi; and combination therapies such as nifurtimox or benznidazole with posaconazole, ravuconazole or amiodarone hydrochloride.

INTRODUCTION

American trypanosomiasis, commonly known as Chagas' disease in honor of the Brazilian physician Carlos Chagas who described it a century ago (1), is a chronic parasitosis caused by the kinetoplastid parasite *Trypanosoma cruzi* that has afflicted humanity since its earliest presence in the New World (2) and is still the largest parasitic disease burden of the American continent (3-5). The disease is technically a zoonosis, as the natural reservoirs of *T. cruzi* are a large variety of marsupial and placental mammals autochthonous to the American continent, and the parasite is naturally transmitted by hematophagous reduviid insects. Human disease results from the invasion of natural ecotopes, as well as from the establishment of the vectors in human dwellings, due to the poor socioeconomic conditions of most rural human populations from Mexico to Argentina, where the disease is endemic (5-7). The parasite can also be transmitted by transfusion of contaminated blood and congenitally from infected mothers to newborns. These routes of transmission, together with intense international migrations during the last 15 years, have led to the spread of the disease to nonendemic areas, such as the U.S. and Western Europe (8-11).

Given its zoonotic character, Chagas' disease is not eradicable (12) but significant advances have taken place in the control of the vectorial and transfusional transmission of the disease in some parts of the continent, particularly by the Southern Cone initiative, leading to a significant drop in the prevalence (from 18-20 million in the early 1990s to ca. 10 million in 2006) and the population at risk (from 100 to 40 million in the same period) (4, 13). Nevertheless, the disease is far from being controlled, due to the uneven extent and quality of control programs in other parts of the continent and limitations of both diagnostic methods and currently available specific treatments (3, 5, 12, 14). Also, in recent years, there has been a worrisome increase in outbreaks of orally transmitted Chagas' disease (15-17), a serious presentation of this condition that could become frequent in an increasingly urbanized Latin America, including the Amazonian region. Unfortunately, due to the complexity of the interactions between this sophisticated parasite and the immune system of its mammalian hosts, an effective prophylactic vaccine has yet to be developed, but some recent results seem promising (18).

This article will discuss the nature and origins of the limited therapeutic approaches currently available for this condition, the reasons for the abysmally low specific treatment coverage levels, currently estimated at < 1% (19, 20), as well as new approaches based on recent advances in our knowledge of the biochemistry, physiology and genetics of the etiological agent and the growing understanding of the pathogenesis of the disease.

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CURRENTLY AVAILABLE DRUGS FOR THE SPECIFIC TREATMENT OF CHAGAS' DISEASE

Limitations and controversies on their application

Chagas' disease is a complex condition resulting from the invasion and successful establishment of the intracellular parasite *T. cruzi* in key tissues of its mammalian host. In humans, the initial acute phase causes a low (< 10%) mortality and generally mild and unspecific symptoms; macrophages, interferon gamma (IFN-γ), $CD4^+$ and $CD8^+$ T helper 1 lymphocytes are the key elements controlling parasite replication (21-23). This acute phase is followed by a lifelong chronic condition, in which the cellular immune response limits the parasite's proliferation but is unable to eradicate the infection, leading to a sustained inflammatory response that underlies the development of one or more of the symptomatic chronic forms of the disease in 30-40% of patients, including chronic Chagas' cardiomyopathy (CCC), digestive problems and neuropathies (22, 24, 25). The most severe of these manifestations is CCC, which typically appears decades after the initial infection and may result in cardiac arrhythmias, ventricular aneurysm, congestive heart failure, thromboembolism and sudden cardiac death. It is the leading cause of cardiac disease and cardiac death in poor rural and urban populations of rural origin in Latin America (24, 26, 27).

The compounds currently available for the specific treatment of this parasitosis are nifurtimox (NFX, Lampit®; Bayer) and benznidazole (BZN, Rochagan®, Radanil®; Roche), which were developed empirically and registered in the late 1960s and early 1970s, originally to treat acute *T. cruzi* infections (Fig. 1). Numerous clinical studies have shown that both drugs have significant activity in congenital and adult acute infections (> 95 and 60-80% of parasitological cures, respectively, as defined by negativization of all parasitological and conventional serological tests [28-30]), but their efficacy varies according to the geographical area, probably due to differences in drug susceptibility among different *T. cruzi* strains (31-33).

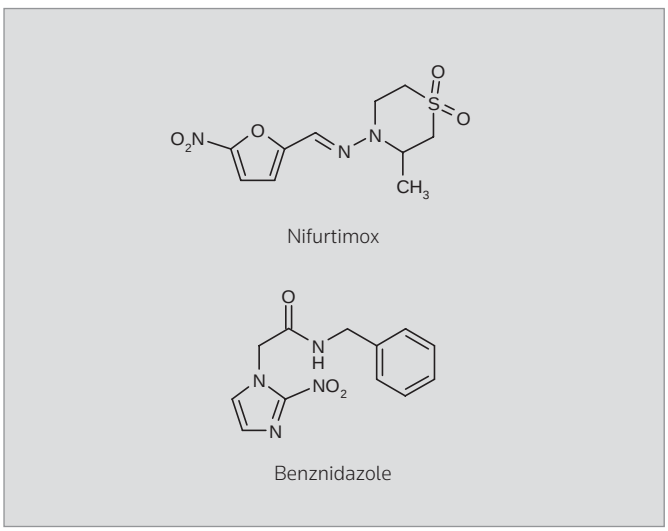

Figure 1. Chemical structure of the drugs currently available for the specific treatment of Chagas' disease: nifurtimox (a 5-nitrofuran) and benznidazole (a 2-nitroimidazole).

Both through experimental infection of animals and in infected humans, the interleukin-12 (IL-12)/IFN-γ axis of the immune system has been shown to play an essential role in the drug-induced parasitological cure (34, 35). Moreover, during the last 15 years, BZN has demonstrated significant curative activity in early chronic infections, with 60-70% radical parasitological cures observed among children up to 14 years of age in Brazil and Argentina (36-38), although other studies on patients in the same age range were unable to detect these high cure rates (39-41). However, the major limitation of NFX and BZN is the drugs' limited antiparasitic activity in the established chronic form of the disease, the most prevalent clinical presentation, as ≥ 80% of treated patients are not parasitologically cured according to the classical criteria indicated above for acute infections (28-30). These results have now been confirmed using recently developed polymerase chain reaction (PCR)-based and special serological methods in both humans and experimental animals (42-51).

The reasons for the marked difference in the antiparasitic efficacy of nitroheterocyclic compounds in the acute and chronic stages of the disease are unclear (28), but they may be related to unfavorable pharmacokinetic properties, such as relatively short terminal half-life and limited tissue penetration (52), which will limit their activity in chronic infections when the parasites are mostly confined to deep tissues and undergo slow replication (52, 53). Nevertheless, several observational clinical studies have shown that chronically infected patients subjected to antiparasitic BZN therapy had a significant reduction in the occurrence of electrocardiographic changes and a lower frequency of deterioration of their clinical condition (38, 54, 55), although they were not parasitologically cured, and other studies do not confirm these findings (38, 46).

Additionally, both NFX and BZN have unwanted side effects that can lead to treatment discontinuation and are related to their mechanism of action, i.e., generation of nitroreduction intermediates that lead to oxidative stress (NFX) or reductive stress (BZN) (56, 57). NFX's side effects include anorexia, nausea and vomiting, causing severe weight loss, insomnia, irritability and (less commonly) peripheral polyneuropathy, while BZN's most common adverse effects are allergic dermopathy, gastrointestinal syndromes and, less frequently, depression of bone marrow, thrombocytopenic purpura and agranulocytosis, polyneuropathy, paresthesia and polyneuritis of peripheral nerves. The incidence of such side effects is variable, depending on the age of the patient (less frequent in younger patients), geographic region and the quality of the clinical supervision of the treatment (30).

On the other hand, long-held ideas on the pathogenesis of the disease have discouraged the specific treatment of chronically infected patients who, as indicated above, are by far the majority of the infected population. Thus, although the role of *T. cruzi* in the pathology of the acute phase of Chagas' disease and the importance of etiological treatment in that stage are widely accepted (22, 30), the participation of the parasite in the pathogenesis of chronic Chagas' disease has been the subject of decades of controversy (21, 24, 52, 56). Several studies implicated autoimmune phenomena as a primary factor leading to the persistent inflammation associated with the pathological manifestations of chronic Chagas' disease, including CCC. This hypothesis was based on the apparent absence of

parasites in the characteristic inflammatory lesions of the heart and gastrointestinal tract associated with symptomatic chronic Chagas' disease and the presence of "antiself" antibody responses in Chagas' disease patients, the latter postulated to result from molecular mimicry between parasite antigens and host cellular components (58, 59). According to such a hypothesis, after the parasite triggers the autoimmune response in the host, its persistence should not play a pivotal role in the pathogenesis of the disease, and even a successful antiparasitic treatment may not lead to an improvement of the patient's clinical outcome. In fact, for decades, the autoimmune hypothesis of chronic Chagas' disease pathogenesis stalled the development of new specific therapeutic approaches for this disease, as antiparasitic treatment in the chronic stage was considered irrelevant (52, 60, 61). This notion, together with the limited efficacy of currently available drugs for long-term chronic infections, has also been one of the main factors responsible for the low treatment coverage of this condition (5).

However, the autoimmune hypothesis has been strongly challenged by the results of many studies in the last 15 years, which have consistently concluded that the persistence of parasites, coupled with an unbalanced immune response in some individuals, leads to the sustained inflammatory responses underlying the characteristic lesions of chronic Chagas' disease (5, 24, 56). In contrast with previous notions, this new paradigm indicates that eradication of *T. cruzi* may be a prerequisite to arrest the evolution of Chagas' disease and avert its irreversible long-term consequences, implying that this condition must be treated primarily as an infectious disease and not as an autoimmune condition (5, 24, 26, 52, 62). Seen from this new perspective, the positive effects of NFX and BZN on patients' clinical evolution, despite their inability to eradicate the parasite, have been explained by drug-induced reduction of the parasite load in infected tissues, which presumably reduces the severity of the associated inflammatory processes (5, 54, 55, 62).

In 1998, the consensus for the specific treatment of Chagas' disease with BZN and NFX was summarized by a group of experts as a set of guidelines (Pan-American Health Organization/World Health Organization, document OPS/HCP/HCT140/99), based on the clinical experience and the level of understanding of the pathogenesis of the disease at that time. Treatment was recommended for acute cases and recent chronic infections, such as those of seropositive children up to 14 years of age, while the treatment of chronically infected adults (with or without cardiac or gastrointestinal involvement) was considered optional. A new consensus based on the parasite persistence hypothesis is currently emerging, indicating treatment of all seropositive patients (including chronically infected adults, as long as no advanced heart disease is present) to reduce or eliminate their parasite loads (8, 26, 54, 63). However, it must be stressed that many physicians still have strong reservations concerning the use of NFX and BZN in chronically infected patients, due to their questionable benefit–risk ratio. Aiming to clarify this issue, a large-scale, randomized, placebo-controlled clinical study is currently under way to assess the effects of etiological treatment with BZN in patients with chronic Chagas' disease (BENEFIT trial) (64).

NOVEL APPROACHES FOR THE SPECIFIC TREATMENT OF CHAGAS' DISEASE

Challenges and promises

The significant limitations of currently available drugs, particularly for the treatment of chronic infections, in addition to our improved understanding of the pathogenesis of the disease, underscore an urgent need for new approaches to the specific therapy of Chagas' disease to address the needs of millions of infected individuals, previously ignored. A major stumbling block for the evaluation of new drugs or the true efficacy of currently available drugs is the lack of reliable biomarkers of parasitological cure and the modification of parasite loads in chronically infected individuals (5). In human patients with established chronic disease, evaluation of the efficacy of any given drug in terms of clinical response would require several years or decades of follow-up, and conventional serology responds slowly to parasite elimination, with the lag time increasing with the duration of the original infection (5, 28, 65, 66). Moreover, the levels of circulating parasites are often at or below the limit of the most sensitive direct parasitological detection methods, such as PCR (43, 67-71).

Studies carried out in a mouse model of chronic Chagas' disease recently showed that radical parasitological cure (verified by immunosuppression of the treated animals) was consistently associated with the disappearance of specific effector/effector memory (T_E/T_{EM}) T cells and the development of a stable central memory (T_{CM}) response (72). More recent work by the same team in chronically infected adult human patients led for the first time to the identification of fast, specific and correlated T- (T_E/T_{EM}) and B-cell responses resulting from specific antiparasitic treatment (73), the latter being evaluated using a novel multiplex assay that uses a panel of recombinant antigens derived from the vertebrate stages of the parasite (74). If confirmed by independent studies, such responses may become the long-sought surrogate biomarkers for treatment efficacy (19). These advances, together with the accumulated basic knowledge on the biology of *T. cruzi* and the organism's interactions with its mammalian hosts, are leading the development of new approaches to the specific treatment of Chagas' disease (56, 75). In the following sections, those with the highest probability of entering clinical trials in the short or medium term will be briefly discussed.

Ergosterol biosynthesis inhibitors

Like most fungi and yeasts, *T. cruzi* requires specific sterols for cell viability and proliferation in all stages of its life cycle, and the ergosterol biosynthesis pathway has been chemically validated at many different steps (56, 76). However, several studies have shown that commercially available ergosterol biosynthesis inhibitors, which are highly effective in the treatment of fungal diseases (e.g., ketoconazole, itraconazole or terbinafine hydrochloride) have suppressive, but not curative, activity against *T. cruzi* infections in humans or experimental animals, and in most cases fail to stop progression of the disease (76). However, during the past 15 years, new triazole derivatives that are potent and selective inhibitors of fungal and protozoal cytochrome P450-dependent lanosterol 14-α demethylase (CYP51), such as D-0870 (AstraZeneca), posaconazole (SCH-56592; Merck & Co.), ravuconazole (Eisai), TAK-187

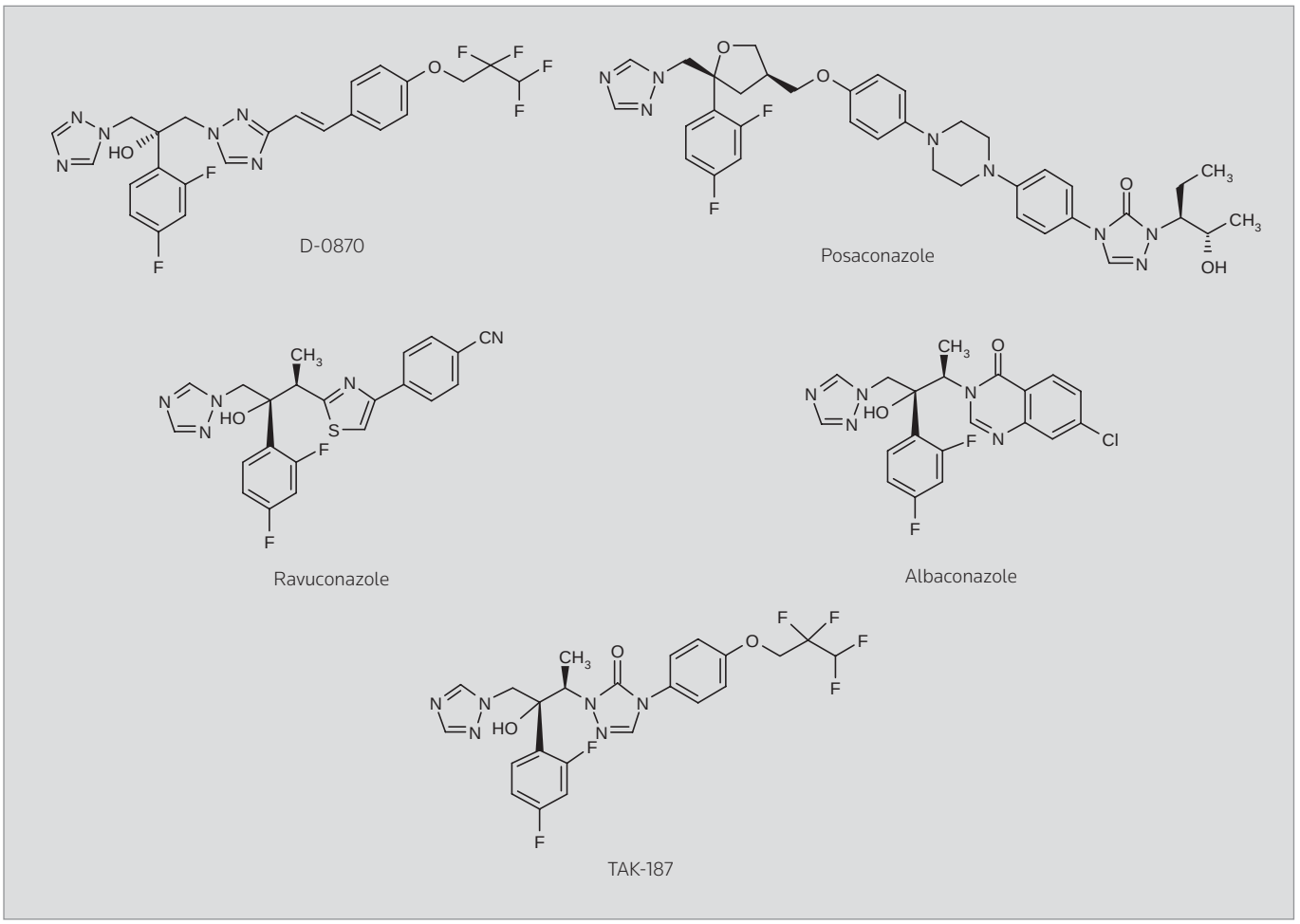

Figure 2. Chemical structures of novel triazole derivatives, specific inhibitors of fungal and protozoan lanosterol 14-α demethylase (CYP51), with curative activity in animal models of acute and chronic Chagas' disease. Posaconazole (Noxafil®; Merck & Co.) and ravuconazole (Eisai) are currently the most advanced candidates for a new anti-*T. cruzi* drug and are poised to enter clinical trials in the short term.

(Takeda) and albaconazole (Uriach), have been shown to induce radical parasitological cure in murine models of acute and chronic Chagas' disease and to be active against nitrofuran- and nitroimidazole-resistant *T. cruzi* strains in vivo, even if the hosts were immunosuppressed (Fig. 2). The remarkable in vivo antiparasitic activities of these triazole derivatives result from a combination of their potent and selective intrinsic anti-*T. cruzi* activity and special pharmacokinetic properties (long terminal half-life and large volumes of distribution) (52, 76).

Among these compounds, the most advanced candidate for new specific treatment for Chagas' disease is the structural itraconazole analogue posaconazole, due to its remarkable antiparasitic potency in vitro and in vivo (56, 76) and its excellent safety profile in humans (77-79). A recent study found that it could eradicate intracellular amastigote *T. cruzi* forms from cultured cardiomyocytes and at the same time allow the full reassembly of the host cells' cytoskeleton and contractile apparatus (80). Furthermore, posaconazole has been shown to be more effective than BZN in reducing the *T. cruzi* load of experimentally infected animals (even in IFN-γ or B-lymphocyte knockout mice [81, 82]), in promoting early trypanocidal immune response in immunocompetent animals and in preventing heart inflammation and damage (83). Finally, earlier this year, a team at the Centre for International Health Research of the University of Barcelona, Spain, presented the first report of parasitological cure with posaconazole of a patient with chronic Chagas' disease compounded by drug-induced immunosuppression, required to control a systemic lupus erythematosus condition (84). The individual had previously been treated with BZN, which reduced the circulating parasite levels but was unable to eradicate the infection. Posaconazole was registered as Noxafil® in 2005-2006 in the E.U., Australia and the U.S. for the treatment and prophylaxis of invasive fungal infections. Based on the findings described above, in 2009, Schering-Plough (now Merck & Co.) announced its commitment to design and execute a clinical trial to evaluate the utility of posaconazole for the specific treatment of chronic human Chagas' disease in collaboration with the Population Health Research Institute (Canada) and a network of Latin American clinical centers (http://www.ifpma.org/index.php?id=2169). This will be the first

rationally designed drug to enter clinical studies for the specific treatment of Chagas' disease.

Ravuconazole has also been shown to be very active against *T. cruzi* in vitro but its in vivo activity in mice was limited, probably due to inadequate pharmacokinetic properties in this animal model (terminal half-life of 4.5 h) (85). Similarly, the activity of ravuconazole in a canine model of acute Chagas' disease was found to be suppressive, not curative, a result that was again attributed to the relatively short half-life of the compound in dogs (8.8 h) (86). However, these results do not necessarily rule out the potential usefulness of this agent in treating *T. cruzi* infections in humans, due to its high intrinsic potency against the parasite (its minimal inhibitory concentration against intracellular amastigotes is 1,000-5,000 times lower than the levels attainable in human plasma with multiple oral dosing) and its remarkably long terminal half-life in man (4-8 days) (87). Thus, ravuconazole, which is currently in phase II clinical trials for the treatment of systemic fungal infections, is yet another candidate for clinical trials in Chagas' disease patients and the Drugs for Neglected Diseases initiative (DNDi) has recently announced that is has reached an agreement with Eisai for the clinical development of E-1224, a water-soluble prodrug (monolysine derivative) of ravuconazole, for the treatment of chronic human Chagas' disease (http://www.dndi.org/ press-releases/532-eisai-and-dndi-enter-into-a-collaboration. html).

TAK-187 is a long-acting triazole derivative with broad-spectrum antifungal activity, which also has very potent anti-*T. cruzi* activity in vitro and is capable of curing both acute and chronic infections in murine hosts, even when the infecting strain is resistant to nitrofuran and nitroimidazole (88). More recent work has shown that it is superior to BZN in preventing cardiac damage in a murine model of Chagas' disease (89). The agent has completed phase I clinical trials (Takeda) and the DNDi has also announced its interest in its clinical assessment in Chagas' disease patients (http://www.dndi.org/component/content/article/12-portfolio/602-azoles-e1224.html).

A different family of azole-based CYP51 inhibitors with potent anti-*T. cruzi* activity in vitro and in vivo was serendipitously discovered in the course of a research program to identify parasite-specific protein farnesylation inhibitors. Such compounds are structurally simpler than the proprietary compounds described above and are an interesting alternative for novel anti-*T. cruzi* drugs (53, 90-93). More recently, a new class of *T. cruzi* CYP51 inhibitors was described, based on the *N*-(4-pyridyl)formamide pharmacophore and derived from a *Mycobacterium tuberculosis* screen hit (94). One of the new agents in this series was able to eradicate intracellular amastigotes grown in cultured mouse macrophages and, in a separate study, was found to have curative activity in a murine model of acute Chagas' disease comparable to that of posaconazole (95). Also, a team at Vanderbilt University has recently described the high-resolution 3D structure of *Trypanosoma brucei* CYP51 with a specifically designed inhibitor at its active site (96). Presumably, the structure of *T. cruzi* CYP51, which has a very high homology with that of *T. brucei,* will be known soon, leading the way to *T. cruzi*-specific inhibitors. Although these new CYP51 inhibitors are still in discovery to lead optimization stages, they could prove to be as potent and even more selective than those previously identified and, coming from nonprofit academic institutions, should be free of the complex intellectual property/price issues of proprietary compounds from the pharmaceutical study.

Amiodarone hydrochloride as an antiparasitic agent

Recently, it was shown that amiodarone hydrochloride (Fig. 3), the antiarrhythmic drug most frequently used in chronic Chagas' disease patients with cardiac compromise (26, 27, 97), also has intrinsic anti-*T. cruzi* activity in vitro and in vivo, and combinations of this drug with posaconazole have synergistic effects (98). It was found that this previously unknown activity results from a dual mechanism of action against the parasite: disruption of Ca^{2+} homeostasis and blockade of de novo ergosterol biosynthesis at the level of lanosterol synthase (oxidosqualene–lanosterol cyclase), which explains the synergistic effects with posaconazole (98). The results suggest that Chagas' disease patients who are being treated with amiodarone may have the added benefit of a reduction of their parasite burden and enhancement of the effects of antiparasitic treatment. This prediction was recently confirmed by a report of a patient with chronic Chagas' disease and advanced cardiac compromise who was treated with a combination of amiodarone and itraconazole, leading to a marked improvement of his clinical condition as well as to parasitological cure, verified by the disappearance of anti-*T. cruzi* lytic antibodies (50, 99). Subsequent work found very similar effects against *Leishmania mexicana* in vitro and in vivo, including a human patient (100-102), indicating a broad antiparasitic action. New clinical trials on the antiparasitic activity of this drug are expected in the near future, alone or in combination.

Cruzipain inhibitors

T. cruzi possesses a cathepsin L-like cysteine protease named cruzipain (cruzaine), which is responsible for the major proteolytic activity of all stages of the parasite life cycle (52, 103). K-777 (CRA-3316), a selective inhibitor of this protease (Fig. 4), blocks the proliferation of both extracellular epimastigotes and intracellular amastigotes and arrests metacyclogenesis (transformation of epimastigotes to metacyclic trypomastigotes) in vitro. In addition, it has been shown to markedly reduce the parasitemia levels and prolong survival in murine models of acute and chronic Chagas' disease (104). A study of the same compound in a canine model of acute Chagas' disease indicated that, although it was unable to cure the infected animals, it significantly reduced parasite-induced cardiac damage (105),

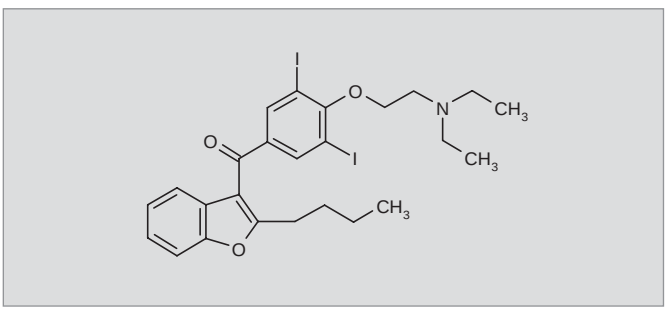

Figure 3. Chemical structure of amiodarone, an antiarrhythmic drug now known to also have potent and selective activity against *T. cruzi.*

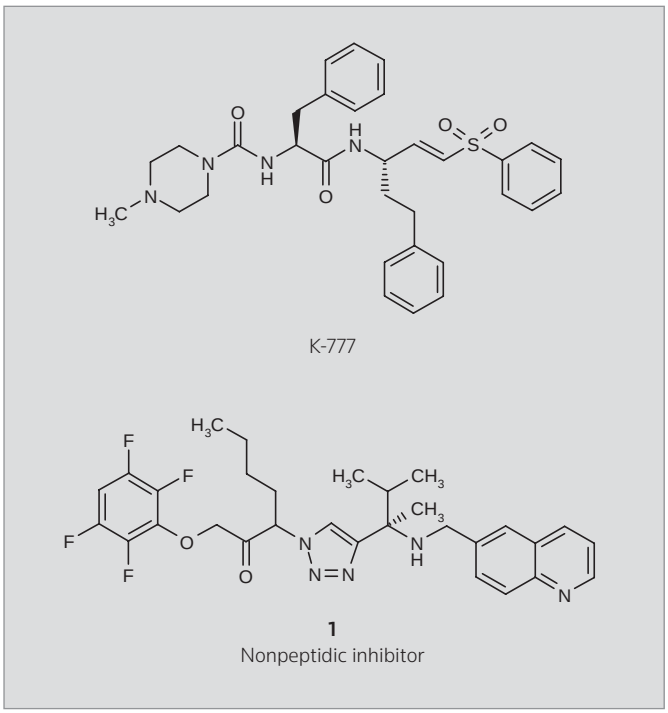

Figure 4. Chemical structures of peptidic and nonpeptidic irreversible inhibitors of cruzipain, an essential *T. cruzi* cysteine protease, validated as a chemotherapeutic target.

while a separate study found it to have curative activity in a murine immunodeficient model of acute Chagas' disease (106). In 2002, Celera Genomics announced that the Institute for One World Health and the National Institutes of Health had initiated development of K-777 as a potential new treatment for Chagas' disease, but in 2005 the Institute for One World Health announced that it was terminating this project, citing hepatotoxicity and serious problems with the agent's manufacture (http://www.oneworldhealth.org/diseases/chagas.php). Although some of these claims have been disputed (107) and K-777 is still considered by its discoverers as the "most advanced inhibitor" of cruzipain (107, 108), no information is publicly available on its clinical development.

In recent years, new lead scaffolds for cruzipain inhibitors have been identified with potent and selective activity against *T. cruzi* in vitro (108-111). Chen et al. (112) have reported the synthesis and characterization of vinyl sulfone-containing macrocycles, synthesized via olefin ring-closing metathesis, which have potent activity against cruzipain and the closely related cysteine protease rhodesain. Structure–activity relationships of nonpeptidic cruzipain inhibitors that are based on the thiosemicarbazone and semicarbazone scaffolds have been described and explained on the basis of the enzyme's known structure and mechanism (110, 113). Also, a new class of nonpeptidic cruzipain inhibitors has been identified, rationally developed by the substrate activity method combined with structure-based design; conversion to inhibitors by the introduction of cysteine protease mechanism-based pharmacophores led to the identification of acyl- and aryloxymethyl ketone cruzain inhibitors, one of which proved to be able to eradicate intracellular *T. cruzi* in irradiated J744 macrophages, with no effects on the host cells (108). Furthermore, a recent report described an initial evaluation of inhibitor **1** in a mouse model of Chagas' disease and further development of the tetrafluorophenoxymethyl ketone class of cruzipain inhibitors (111) (Fig. 4). Taken together, these results indicate that cruzipain is a confirmed target for anti-*T. cruzi* chemotherapy. However, the potential of peptide-like inhibitors (such as vinyl sulfone derivatives) as anti-*T. cruzi* drugs remains unclear, while the newer nonpeptidic inhibitors are still in discovery to lead optimization stages.

Combination therapies

Combined anti-infective therapies have several objectives: 1) to reduce the dose and/or duration of the treatment, with a concomitant reduction in side effects and costs, as well as improvement of the patient's compliance; 2) to exploit potential synergistic effects of concomitant treatments; and 3) to forestall the development of drug resistance by the etiological agent. Such combinations have been used for decades in antifungal, antiparasitic and antiviral therapy, as well as in the treatment of fastidious bacterial infections such as tuberculosis. However, this concept has not yet been incorporated in the specific therapeutic management of human Chagas' disease, despite the limitations of currently available drugs and the long (30-60 days) treatments involved.

From the beginning, the emergence of ergosterol biosynthesis inhibitors as potential anti-*T. cruzi* agents led to interest in the study of drug combinations acting at different steps of the pathway, since their combined action was predicted to have synergistic effects (114). Several studies confirmed this hypothesis, demonstrating strong synergism in the anti-*T. cruzi* action of ketoconazole (a CYP51 inhibitor with suppressive, not curative, anti-*T. cruzi* activity in vivo) combined with the squalene epoxidase inhibitor terbinafine in vitro (115, 116) or with the HMG-CoA reductase inhibitor lovastatin (mevinolin), both in vitro and in vivo (117). Furthermore, it was shown that combinations of 22,26-azasterol or 24(*R*,*S*),25-epiminolanosterol, inhibitors of 24-sterol *C*-methyltransferase (an essential enzyme in ergosterol biosynthesis not present in mammals), with ketoconazole had strong synergistic effects against *T. cruzi* in vitro and in vivo (118). Another study found that combinations of ketoconazole with alkyl-lysophospholipid analogues (inhibitors of *T. cruzi* phosphatidylcholine biosynthesis) had synergistic antiproliferative effects against the parasite in vitro.

On the other hand, an independent study found that combinations of BZN with ketoconazole had synergistic effects in a murine model of acute Chagas' disease (119) and, as described in the previous section, it is now known that the antiarrhythmic drug amiodarone also has anti-*T. cruzi* activity and acts synergistically with posaconazole in vitro and in vivo (98).

Based on these antecedents, drug combinations are being considered for evaluation of their efficacy in both experimental and human Chagas' disease:

- Combinations of NFX or BZN with ergosterol biosynthesis inhibitors, such as posaconazole or ravuconazole
- Combinations of amiodarone with BZN or NFX, and of amiodarone with ergosterol biosynthesis inhibitors (itraconazole, posaconazole or ravuconazole)

- Combinations of ergosterol biosynthesis inhibitors acting at different steps of the pathway, such as CYP51 inhibitors (itraconazole, posaconazole or ravuconazole) with HMG-CoA reductase inhibitors (statins) or squalene synthase inhibitors (120-123)

A report from Bahia and coworkers informs on promising results with the first option in a murine model of acute Chagas' disease, but the data are still very preliminary (124). Similar combinations could be incorporated in the initial clinical trials of posaconazole and ravuconazole, referred to above.

CONCLUSIONS

The therapy of Chagas' disease, a long-neglected disease despite its heavy burden of morbidity and mortality (3, 5, 14), has traditionally received little attention, not only due to the lack of resources and/or political will from many of the governments in the endemic countries and the absence of economic incentives for the for-profit pharmaceutical industry, but also due to long-lasting controversies on the relevance of specific treatment in the prevalent chronic stage of this condition (5, 56). However, the sustained accumulation of knowledge on the biology of the parasite, our better understanding of the pathogenesis of the disease and the growing interest from some governments, product development partnerships, such as DNDi, and several pharmaceutical companies, could lead to clinical testing in the short term of new types of drugs or drug combinations that may radically change the therapeutic management of this condition (12, 56).

Currently, the most advanced candidates for new specific treatment for Chagas' disease are the novel triazole derivatives (CYP51 inhibitors) posaconazole and ravuconazole, the first already registered for the prophylaxis and treatment of invasive fungal infections, and the second in advanced clinical trials for the same application (see above). The special advantages of posaconazole are its excellent safety profile and its superior anti-*T. cruzi* activity over BZN in animal models of acute and chronic Chagas' disease, even when the hosts are immunosuppressed. As indicated above, this superior activity has now been demonstrated in a human patient with chronic Chagas' disease and sustained immunosuppression (84). The agent's main limitation is its difficult and costly manufacture, making its current price too high for its widespread use in endemic countries. Notwithstanding, this issue could potentially be approached through differential pricing, which has been used for other on-patent pharmaceuticals in separate markets (125). On the other hand, compounds such as ravuconazole, with the same mechanism of action and similar pharmacokinetic profile in humans but simpler molecular structure and lower potential cost of goods, could also enter clinical development for this condition in the short term (see above). Moreover, combinations of the new drugs with currently available ones (NFX and BZN) aimed at a reduction of the dose and/or length of treatment are contemplated in upcoming clinical trials and could become the standard chemotherapy for this condition in the future.

The recently discovered anti-*T. cruzi* activity of amiodarone poses intriguing questions on the potential extra benefit of treatment with this drug in patients with chronic Chagas' disease, as together with the expected control of their cardiac arrhythmias, they could have a reduction of their parasite loads. This idea is currently being investigated by retrospective clinical analyses. Combination therapies of this drug or analogues with currently available ones or the new triazoles are also being considered. Although the combination of amiodarone with posaconazole has been shown to act synergistically against *T. cruzi* in vitro and in vivo, care must be exercised when using this compound with others that can prolong the Q-T_c interval (such as fluoroquinolones, macrolide antibiotics or azoles), as it has been reported that such combinations can lead to worsened arrhythmia (98). However, no significant side effects were reported in a patient with chronic Chagas' disease who was treated with the combination of amiodarone and itraconazole for several months (99).

Finally, inhibitors of the cysteine protease cruzipain are also promising candidates for new anti-*T. cruzi* drugs, although much less advanced than CYP51 inhibitors. Again, dual or triple combinations of this class of compounds with currently available drugs or those now entering clinical trials could eventually play a significant role in the treatment of human Chagas' disease, as they do in advanced (highly active antiretroviral) HIV therapy.

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DISCLOSURES

The author states no conflicts of interest.

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