International Journal for Parasitology: Drugs and Drug Resistance 2 (2012) 236–242

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology: Drugs and Drug Resistance

journal homepage: www.elsevier.com/locate/ijpddr

Invited Article

Recent developments in sterol 14-demethylase inhibitors for Chagas disease

Frederick S. Buckner [a,*,1], Julio A. Urbina [b,1,2]

[a] Department of Medicine, University of Washington, Seattle, WA 98195, USA
[b] Emeritus Investigator, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela

ARTICLE INFO

Article history:
Available online 10 January 2012

Keywords:
Trypanosoma cruzi
Chagas disease
CYP51
Sterol 14-demethylase

ABSTRACT

The protozoan parasite, *Trypanosoma cruzi*, causes the most prevalent parasitic infection in the American continent. It gives rise to life-long infection in humans and results in severe cardiomyopathy or other life-threatening manifestations (Chagas disease) in ~30% of those infected. Animal models and clinical studies indicate that etiological treatment of the infection reduces the risk of developing the disease manifestations. Unfortunately, the existing chemotherapeutics have suboptimal antiparasitic activity and cause significant side effects in many patients, thus better anti-trypanosomal drugs are greatly needed. The sterol biosynthesis pathway has received attention as a target for the development of new drugs for Chagas disease. In particular, inhibitors of sterol 14-demethylase (CYP51) are shown to be extremely active on *T. cruzi in vitro* and in animal models. Antifungal drugs (i.e. azoles) in clinical use or in clinical studies have been extensively tested preclinically on *T. cruzi* with posaconazole and ravuconazole demonstrating the most promising activity. As a result, posaconazole and a pro-drug of ravuconazole (E1224) are currently being evaluated in Phase II studies for Chagas disease. Additional CYP51 inhibitors that are specifically optimized for anti-*T. cruzi* activity are in development by academia. These represent an alternative to proprietary antifungal drugs if the latter fall short in clinical trials or are too expensive for widespread clinical use in disease endemic countries. The research over the next few years will help define the role of CYP51 inhibitors, alone or in combination with other drugs, for managing patients with Chagas disease.

1. Introduction to Chagas disease

The etiologic agent of Chagas disease, the protozoan *Trypanosoma cruzi* (*T. cruzi*), is primarily transmitted to humans by blood feeding *Triatomine* bugs. Vectorial transmission is confined to the American continent, whereas infection by blood transfusion or by mother-to-baby transmission occurs wherever individuals harboring this chronic infection reside or immigrate. Approximately 8–10 million people are infected in Latin America making it the most prevalent parasitic disease in the American continent and the first cause of heart disease and heart deaths among poor rural populations in Latin America (Rassi et al., 2010). An estimated 300,000 infected persons live in the United States, mostly immigrants from Latin America. A comparable number are living with this parasite in Europe (Gascon et al., 2010; Leslie, 2011).

T. cruzi infects a wide range of mammalian hosts where it establishes a chronic infection. During the initial acute phase, the parasite rapidly cycles between a replicative intracellular stage (amastigotes) and a non-replicative bloodstream stage (trypomastigotes), successfully disseminating throughout the body. The protozoan is capable of infecting diverse host cell types where it replicates freely within the cytoplasm (as opposed to within a vacuolar organelle, which is the case for the related parasites of the *Leishmania* genus). In an immunocompetent host, the infection is controlled by a mixed immune response involving both humoral and cellular effector mechanisms but, in the large majority of cases, the parasite is not eradicated leading to life-long infections. During the acute stage, lasting just a few weeks, persons have flu-like symptoms, and are rarely diagnosed or treated; this stage has low (ca. 5%) mortality and leads to an initially asymptomatic (“indeterminate”) phase, which for 60–70% of infected persons lasts for the rest of their lives. In the remaining ~30–40% of individuals, chronic Chagas disease manifests within 1–3 decades, primarily involving the heart, gastrointestinal tract, or nervous system. Chagasic cardiomyopathy, which results from a chronic inflammatory process triggered and sustained by the persistence of the parasite (Urbina, 2010; Marin-Neto et al., 2007; Rassi et al., 2010), is associated with malignant arrhythmias, embolic events, and/or rapidly progressive congestive heart failure and death. Since persons are often infected as children, the morbidity and mortality from Chagas disease typically strikes during the prime adult years in people’s lives.

* Corresponding author. Tel.: +1 206 616 9214; fax: +1 206 685 6045.
E-mail addresses: fbuckner@u.washington.edu (F.S. Buckner), jurbina@mac.com (J.A. Urbina).
[1] Contributed equally.
[2] Current address: 734 Springhill Lane, Cincinnati, OH 45226, USA. Tel.: +1 513 321 2981.

2211-3207
doi:10.1016/j.ijpddr.2011.12.002

Two clinical drugs exist for treating Chagas disease, both developed empirically over 40 years ago. These are nitroheterocyclic compounds, benznidazole and nifurtimox, that act by generating free radicals or reductive stress in *T. cruzi* cells. When used during acute infection, they cure up to 80% of infections, but the vast majority of patients are not diagnosed until they are in the chronic stage where, unfortunately, parasitological cure rates with the drugs, as assessed by conventional serology, are less than 20%. Many clinicians are reluctant to use these drugs because of the unfavorable risk-to-benefit profiles due to side effects such as allergic dermopathy, vomiting, psychosis, and neuropathy (Urbina and Docampo, 2003; Urbina, 2010). Clinical data and animal models indicate that parasitological cures or reduction of the parasite burden of the patients are associated with improved clinical outcomes (Viotti et al., 2006, 2011). As a consequence, new drugs with greater antiparasitic activity and improved safety profiles would potentially make it possible to treat the scores of individuals harboring both acute and chronic *T. cruzi* infections, to prevent or mitigate the manifestations of chronic Chagas disease.

2. Sterol biosynthesis and CYP51 of *T. cruzi*

Sterols are essential lipid components of eukaryotic membranes. These molecules are important regulators of membrane physical properties, such as permeability and fluidity, and also have essential roles in aerobic metabolism, regulation of cell cycle, sterol uptake, and sterol transport (Daum et al., 1998). The initial steps in cholesterol biosynthesis also lead to the synthesis of other important molecules, including dolichol, ubiquinone, isopentyladenine, heme A, and prenylated proteins. The final products of sterol biosynthesis vary among eukaryotes with mammals producing cholesterol, while fungi plants and protozoa produce 24-alkyl sterols, with distinct modifications of both the steroid nucleus and the alkyl side chain for each phylogenetic group. The sterol biosynthesis pathway of *T. cruzi* epimastigotes is illustrated in Fig. 1. *T. cruzi* is similar to fungi in its sterol composition, with ergosterol (24-methyl-5,7,22-trien-3β-ol) and its 24-ethyl analog (24-ethyl-cholesta-5,7,22-trien-3β-ol) being the major mature sterols in the epimastigote stage (within the insect host) (Fig. 1) (Furlong, 1989; Korn et al., 1969; Urbina et al., 1998; Liendo et al., 1998). The major sterols produced by the amastigote stage (inside the mammalian host cells) are fungisterol (ergosta-7-en-3β-ol) and its 24-ethyl analog (24-ethyl-cholesta-7-en-3β-ol) (Liendo et al., 1999) (Fig. 2). Although *T. cruzi* incorporates its mammalian host sterols (primarily cholesterol) into its membranes, it has an essential requirement for *de novo* sterol synthesis for survival in all stages of its life cycle and is highly susceptible to sterol biosynthesis inhibitors (Liendo et al., 1998, 1999; Urbina et al., 1998).

Synthesis of the major animal sterol (cholesterol) and the 24-alkyl-sterols in fungi, protozoa and plants, requires removal of the 14α-methyl groups from sterol precursors. The reaction is catalyzed by a microsomal cytochrome P450, the sterol C14-demethylase (CYP51). In mammals and yeast, where the substrate is lanosterol (Fig. 1), the enzyme is frequently called the lanosterol 14α-demethylase. In *T. cruzi*, new data suggests that the preferred substrate is not lanosterol, but rather eburicol (24-methylene-dihydrolanosterol) (Lepesheva et al., 2006) (Fig. 1). CYP51 specifically catalyzes the removal of the C14 methyl group from the sterol scaffold through three successive oxidations resulting in decarboxylation, releasing formic acid (Fischer et al., 1989). During catalysis, the active-site heme iron is reduced by a P450-reductase enzyme utilizing NADPH, from the resting ferric (Fe^{+++}) state to active ferrous (Fe^{++}) state; the resting state is regenerated in each cycle by the transfer of electrons to oxygen and the incorporation of this atom in the C14 substituent of the sterol substrate (Lepesheva et al., 2011). Inhibition of cytochrome P450 enzymes by azole drugs (discussed below) results from coordination of the azole nitrogen to the heme iron, with the lipophilic ligand attached to the azole occupying the binding site for the substrate. These inhibitors prevent both binding of the substrate and oxygen activation (Walker et al., 1993). Recent crystal structures of trypanosomal CYP51 bound to substrate and inhibitors have provided news insights into the details of the catalytic site of this enzyme (Chen et al., 2010; Lepesheva et al., 2010a,b; Hargrove et al., 2011).

3. History of azole drug testing on *T. cruzi*

Azole derivatives with selective activity against fungal CYP51 (devoid of significant activity against the human ortholog enzyme) have been used as first line antifungal drugs since the 1970s. The first description of activity of CYP51 inhibitors on *T. cruzi* was reported by Docampo and colleagues thirty years ago (Docampo, 1981). This report described growth inhibitory activity and ultrastructural alterations on *T. cruzi* cells produced by the topical antifungal azoles miconazole and econazole. Numerous studies followed over the years with the theme of testing antifungal CYP51 inhibitors against *T. cruzi*. The earlier generation imidazoles (e.g. ketoconazole, miconazole, clotrimazole) and triazoles (e.g. fluconazole and itraconazole) were found to have potent and selective *in vitro* activity, but were not curative in animal models of *T. cruzi* infection (Buckner, 2008). Referred to collectively as "azoles", these drugs have profound effects on the sterol composition of cultured *T. cruzi* indicating that their mechanism of action is through disruption of sterol biosynthesis, presumably by binding to *T. cruzi* CYP51, since the block results in accumulation of 14-methylated sterols (Urbina et al., 1996, 1998; Liendo et al., 1999). Additional studies have shown direct binding of these compounds to purified recombinant *T. cruzi* CYP51 as well as inhibition of its enzymatic function *in vitro* (Buckner et al., 2003; Lepesheva et al., 2007).

As new azole drugs have been developed by pharmaceutical companies to combat fungal diseases, these in turn have been tested for activity against *T. cruzi*. The experimental azole drug, D0870 was the first to show cure of mice chronically infected with *T. cruzi* (Urbina et al., 1996). Unfortunately, D0870 was discontinued in clinical trials (of fungal infections) due to untoward side effects (Williams and Denning, 2001). The second-generation azole antifungal drug, voriconazole, has comparatively weak anti-*T. cruzi* activity (Buckner, 2008) and has not been pursued for clinical development. Posaconazole (Noxafil®, Merck; Fig. 3), on the other hand, is the most potent and efficacious azole drug against *T. cruzi* yet to be identified (Liendo et al., 1999; Urbina et al., 1998; Molina et al., 2000). Clinically approved in 2006 for the prophylaxis and treatment of invasive fungal infections due to its broad-spectrum antifungal activity and excellent safety profile, posaconazole could potentially be repurposed for use in Chagas disease, thereby avoiding substantial costs and risks of developing a new chemical entity. It is the only clinically approved azole drug that is curative in the chronic murine model of *T. cruzi* infection and has potent activity against benznidazole- and nifurtimox-resistant *T. cruzi* strains, even in immunocompromised animals (Molina et al., 2000). The *in vitro* potency of posaconazole (subnanomolar concentrations against intracellular amastigotes) is probably due to its very tight binding to *T. cruzi* CYP51, which involves direct interactions with 13 amino acid side chains in the active site and 12 in the hydrophobic substrate access channel and has, in fact, a stabilizing effect on the tertiary structure of the protein (Fig. 4) (Lepesheva et al., 2010a; Chen et al., 2010). These facts, combined with the favorable pharmacokinetic characteristics (including large volumes of distribution and long terminal half life) of the drug in humans and