Tricyclic drugs: a possibility for Chagas disease treatment

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Summary
Chagas disease affects about 18 million people and 25% of the population of Latin America is at risk of acquiring Chagas disease. The chemotherapy of Chagas disease is still an open field and remains as an unsolved problem. Nifurtimox and benznidazole are currently used to treat this disease, however, both drugs have high toxicity, are mutagenic and patient gives up treatment.

T. cruzi enzymes such as trypanothione reductase, represent potential drugs targets because they play an essential role in the life of this organism. This enzyme has been isolated, purified and studied by X ray cristalography. Phenothiazines and related compounds inhibit trypanothione reductase and a specially favoured fit is a small 2-substitued 2-chloro and 2-trifluoromethyl with a remote hydrophobic patch. The essential phenothiazine nucleus can adopt more than one inhibitory orientation in its binding site.

Phenothiazines and related compounds are drugs used in psychiatric treatments, that besides its action upon trypanothione reductase through the peroxidase/H$_2$O$_2$/system, also exert other trypanocidal effects upon epimastigotes and tripomastigotes forms: clomipramine through an anticalmodulin action; trifluopherazine and thioridazine induced disruption of mitochondria and prometazine provoked serious cell membrane disorganization. Clomipramine and thioridazine were also effective in treatment of mice with experimental Chagas disease, since it proved to modify significantly the natural evolution of the infection; cardiac function and survival of infected and treated animals were not different from non infected. Phenothiazines and related compounds are promising trypanocidal agents for treatment of Chagas disease.

Introduction
Chagas' disease has a wide distribution in Central and South America and is endemic in 21 countries. It is caused by a flagellate protozoan parasite, the Trypanosoma cruzi, principally transmitted to human by a blood-sucking triatomine bug (Triatoma infestans) that finds a favourable habitat in crevices in the walls and roofs of poor houses in rural areas and peripheral urban slums. Humans and a large number of species of domestic and wild animals constitute the reservoir.

This disease affects between 16 and 18 million people and 25% of the population of Latin America is at risk of acquiring Chagas disease [1,2,3]. There are three stages of human disease [4,5]: the acute phase which is short, the indeterminate phase which is a silent period that may last between 10 to 20 years. After this becomes the chronic phase in which 30% of the infected people develop irreversibly compromise of heart which may led to sudden death, 6% present digestive organs dysfunction and 3% peripheral nervous system disturbances [5].

Chronic Chagas heart disease is a cardioneuropathy in which sympathetic and parasympathetic systems are affected. It has been described antibodies with reactivity against cardiac receptors that trigger signal transduction inducing physiological, biochemical and pharmacological alteration of cardiac function [6,7,8].

The β-adrenergic receptors-G-protein-adenyl cyclase system is the most powerful physiologic mechanism to increase contractility [9], so the β receptors’ studies are giving information about the inotropism of heart.

We have studied cardiac β receptors’ function analysing their affinity and density in hearts obtained from T. cruzi infected mice. They have demonstrated to be very good indicators of Chagas’ disease phases, because their alterations are very different in each moment of the infection [10-13]. Besides other authors have also described cardiac β adrenoceptors alterations in cardiac tissues from chagasic mice, and associate cardiac dysfunction with the presence of circulating antibodies against these receptors [14].
After several decades of investigations the pathophysiology of Chagas heart disease is not completely understood [15,16]. Two primary hypothesis are proposed to account its pathogenesis: that the persistence of *T. cruzi* at specific sites in infected host results in chronic inflammatory reactivity [17] and that *T. cruzi* infection induces immune response which are targeted at self tissues. [18,19].

The main support for the autoimmune hypothesis is the conclusion that signs of the disease are present in tissues with apparent absence of *T. cruzi*. This observations suggest that the inflammatory lesions are induced by self cross-reactive or mimicked antibodies [15,20]. Antibodies anti cardiac beta adrenergic and muscarinic receptors would seem to play an important role in the pathogenesis of bradyarythmias and tachyarrythmias which originate the disorders in the chagasic cardiopathy. It has been reported the existence of circulating IgG in chagasic patients and in experimental model, which react with β1 and β2 adrenoceptors [14].

The view that the parasite persistence is the cause of heart disease has been demonstrated in the findings of Jones et al [21] that reported *T. cruzi* DNA in hearts of patients with chagasic cardiomyopathy.

Other pathophysiology mechanism proposed is the coronary microvascular alterations that may lead to myocardial damage and dysfunction through different mechanisms, including myocardial ischemia. This microvascular hypothesis has received strong support from experimental and clinical observations [22,23].

The autoimmunity and parasite persistence hypotheses suggest very different directions for the treatment: advocates for autoimmune hypothesis propose to treat only acute phase because the *T. cruzi* is surely present. In contrast the parasite persistence hypothesis would support the view of treatment at any moment of infection [7,24].

The chemotherapy of Chagas' disease is still an open field and remains as an unsolved problem. Only two nitroheterocyclic drugs, nifurtimox and benznidazole are in clinical use and currently used to treat this disease. They inhibit DNA, RNA and protein synthesis. They also increment macromolecules degradation [25-28]. Redox- cycling of both drugs generate "active oxigen species" which contribute to explain their trypanocidal effects [29-32].

However, both drugs have high toxicity so their side effects are not desirable, patient gives up the treatment, are mutagenic [33-35] and produce biochemical damages in mammalian tissues [36,37]. Also, the genetic structure of the parasite [38], leads to the frequent appearance of strains more resistant to the treatment.

For this there is a real need for investigations about other drugs effective and less toxic for Chagas' disease.

Particularly important for this purpose is the knowledge of the parasites biochemical pathways which facilitate the search for drugs, because it involves identification of a drug target, its isolation and detailed characterization of its molecular and kinetic properties, the identification of inhibitors and their optimization to improve their pharmacological and toxicological properties [39].

Cruzain (cruzipain) is a *T. cruzi* cystein protease described first by Cazzulo et al [40], and then crystallized [41] and proposed as target for the design of antiparasitic drugs [42]. Trypanothione reductase is also a *T. cruzi* enzyme [43] that has progressed as a chemotherapeutic target.

**Trypanothione Reductase**

The mammalian redox defense system, based on glutathione oxidiced (GSSG) and glutathione reductase (GR) is replaced in trypanosomes and *Leishmania* by an analogous, but distinct, system based on oxidiced trypanothione and trypanothione reductase (TR) [44].

Living cells rely on two classes of low molecular mass chemicals, polyamines and thiol-containing compounds, in a wide range of biological functions. Among others, polyamines are implicated in protein synthesis, cell growth and development [45], whereas the peptide glutathione (L-α-glutamyl-L-cysteinylglycine) is involved in maintaining redox balance and the regulation of diverse aspects of metabolism. The thiol form of glutathione, GSH, functions as a protective agent, maintaining an intracellular reducing environment. GSH is oxidiced to glutathione disulfide (GSSG), reaction with potentially damaging radicals and oxidants.

The enzyme glutathione reductase (GR) ensures that high thiol levels are preserved by catalyzing the reduction of the disulfide. The GR-GSSG system has been thoroughly investigated by a variety of techniques, including crystallography [18].
Trypanosomatids use polyamine-glutathione adducts, instead of GSH, to function in similar protective and regulatory roles [46]. Trypanothione itself performs a variety of functions: ascorbate homeostasis, thiol/disulfide exchanges, conjugation of metal and drugs, synthesis deoxiribonucleotides and reduction of hydroperoxides [47,48] which in other organism, are fulfilled in different ways.

Similarities with glutathione suggested that these metabolites might have a similar biological function in scavenging free radicals and oxygen-reactive species formed by metabolic processes or when the parasites are subjected to oxidative stress by the host immune response.

Trypanosome contain low levels of glutathione but do not possess GR. The enzyme trypanothione reductase is responsible for the conversion of trypanothione disulfide. In the following figure the Trypanothione reductase system is shown.

![Trypanothione reductase system](image)

Human GR and TR are both NADPH-dependent, flavin-containing disulfide oxidoreductases. They function as homodimers of subunit molecular mass in the range 52-54 kDa and share about 30% sequence identity. The key residues involved in catalysis are conserved but the critical observation has been made that each enzyme is specific for its cognate substrate, despite mechanistic and structural similarities [49,50].

This indicates that it should be possible to inhibit the parasite TR but not the human host GR.

Selecting a probable drug target molecule, necessitates proving that the enzyme is essential for the organism in question. However, in most cases, an effective and highly specific inhibitor is not available and so interactions with other cell components cannot be excluded. Therefore, genetic approaches have become accepted as the more reliable tools.

**Trypanothione Reductase Inhibitors**

Phenothiazines and related compounds are tricyclic drugs used in psychiatric treatments as antidepressant, anxiolytic and antipsychotic. They possess ability to cross the blood-brain barrier and to accumulate in brain, provoking specially an effective dopamine receptors blockade. They also have antihemetic and antihistaminic effects. Among their different biological activities some phenothiazines and related compounds have potent antifungal, antibacterial and antiplasmodial activity that can be applied in elimination of drug resistance of bacteria [51,52]. This drugs also showed antitumor and immunomodulation activity [53-56]. They also interact with membranes or their compounds [57] with cellular proteins [58], with dopaminergic receptors, [59-61], inhibit the activity of Mg2+-dependent ATP-ase, increase the membrane fluidity and present a marked anticalmodulin action [62]. Besides, a lethal effect upon *Leishmania donovani* have been also observed [63].

Trypanocidal effect of some phenothiazines and related compounds upon different *T. cruzi* stages (epimastigotes and tripomastigotes) were studied in our laboratory [64]. These works demonstrated that clomipramine lethal effect can be achieved to its anticalmodulin action; trifluoperazine induced disruption of parasite mitochondria and prometazine provoked serious cell membrane disorganization [65-67]. Thioridazine had a direct trypanocidal effect, provoking mitochondrion and kinetoplast disorganisation in tripomastigotes and condensation of cytoplasm organoids close to plasmatic membrane in epimastigotes; membranes and flagello alterations were not detected in neither of the *T. cruzi* stages studied [67].
Phenothiazines and related compounds with substituents in positions 2 and 10 exhibit a number of interesting analytical properties due to their characteristic structure. Of these properties the most important are the liability to oxidation by means of many oxidizing agents.

Trypanothione reductase is irreversibly inhibited by peroxidase/H$_2$O$_2$/phenothiazine systems. Trypanothione reductase inactivation depended on time of incubation with phenothiazines system, the peroxidase nature and the phenothiazine structure and concentration. Production of phenothiazines cations radicals by phenothiazines peroxidation was essential for the enzyme inactivation [68]. Phenothiazines are direct inhibitors of *Trypanothione reductase* [69] and the actions of peroxidase-activated phenothiazines through three different peroxidases were studied by Gutierrez-Correa et al [68]: horseradish peroxidase, myeloperoxidase and a Mb, an heme protein which is a dioxigen stabilizer in striated muscle. Their results support the hypothesis that cation radicals produced by phenothiazine peroxidation irreversibly inhibited the *T. cruzi* enzyme. These works explain the benefit effect of phenothiazines and related compounds found in modifying the evolution of experimental Chagas’ disease.

**Clomipramine and Thioridazine Modified the evolution of Experimental Chagas’ Disease**

Clomipramine and thioridazine are used in clinical treatments for their antidepressant and tranquilliser effects respectively. They present a potent blockade effect in dopaminergic receptors [70] and also belong to the drugs that are able to inhibit trypanothione reductase and also calmodulin by binding to the CaM-recognition site [16]. They also present trypanocidal effects “in vitro” studies [65,68]. Gutierrez-Correa et al [69] demonstrated the capacity of different tricyclic drugs to inhibit the activity of trypanothione reductase, see Table I.

This helps to explain our results using thioridazine or clomipramine [71,72] as treatment for mice infected with low number of tripomastigotes of *T. cruzi*, in the acute, indeterminate and chronic stages of Chagas disease [73,74,58]. The effectiveness was verified studying parasitaemias, survival, electrocardiography histopathology and affinity and density of cardiac beta receptors.

Cardiac β receptors functions are modified in a particular manner in each *T. cruzi* infection phase [75,11,12]: increase in the binding sites number and a decrease in the affinity values, were observed in the acute phase of the infection, showing that the regulating mechanism between affinity and density was kept. In the indeterminate stage a significantly reduction in cardiac receptors affinity and density similar to the described in the acute phase were detected; instead a marked decrease of both parameters characterised the chronic stage. These results are indicating different degrees in cardiac function alteration. Viscosity and fluidity of cardiac membrane were also analysed finding values similar to normal in any of the stages studied.

Treated mice presented lower parasitaemias and became negative by day 30 using either of the drugs. In the chronic phase 80% of untreated mice were dead, but 80% of treated survived at this time. Two years later these mice were alive.

Low frequency and mild electrocardiographic abnormalities (prolonged PQ segment and QRS segment) were detected in treated groups, p< 0.001 than infected (See Table II). Hearts from treated mice showed minor inflammatory infiltrates in the acute phase, but none structural abnormalities were detected in the chronic phase. Treated animals had significantly lower cardiac beta receptors affinity (Kd (nM)): Clomipramine 4.56±0.98, Thioridazin 5.48±0.21 and higher density (Bmax (fmol/mg protein)): Clomipramine 65.01±4.88, Thioridazine 72.34±1.06 when compared with untreated infected mice (Kd (nM) 11.21±0.26, Bmax (fmol/mg protein) 53.33±0.71, (p<0.01). Values from treated animals were not different from those of uninfected animals (Kd: 3.61±0.05, Bmax 71.97±0.36) except for thioridazine treated mice, that the affinity remained higher, showing that a compensatory mechanism was taking place and that cardiac function was conserved (See Table II). Viscosity and fluidity of cardiac membrane remained unaltered.

Both tricyclic drugs are in clinical use, for this the results are interesting because can be the basis for new agents for the treatment or prevention of Chagas disease.

Trifluoperazine and promethazine provoked trypanothione reductase inactivation of 37% and 26% respectively [68] after an incubation time of 30 minutes (see Table I). Our results with these drugs were satisfactory upon epimastigotes and tripomastigotes “*in vitro*” [67,64]. Mice infected and treated with either drugs improved their parasitaemias, but the survival was only one month superior than non treated animals, showing that the poor inhibitory mechanism of the *T. cruzi* enzyme of these compounds explain the little lethal effect upon the parasite “*in vivo*”.

The conclusion is that not all phenothiazines and related compounds have the same capacity to inhibit...
trypanothione reductase, and this has a direct relationship with the effectiveness of these drugs as treatment of experimental Chagas disease.

**Final Considerations**

Over the last two decades, progress towards new drugs for the treatment of Chagas disease has been disappointing [76]. However chemotherapeutic targets have been identified either through comparative biochemistry (ergosterol), comparative biology (kinetoplast) or through studies of the mode of action of experimental compounds or drugs (trypanothione) [77].

Trypanothione reductase has been widely identified as a drug target, has been isolated and detailed characterised in its molecular and kinetic properties. Clomipramine and Thioridazine are drugs in clinical use and have demonstrated to inhibit trypanothione reductase and to modify experimental Chagas disease evolution, for this phenothiazines and related compounds are promising trypanocidal agents for the treatment of Chagas disease.

**References**


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