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Chapter

Translational Research on Chagas Disease: Focusing on Drug Combination and Repositioning

Marcos André Vannier-Santos, Ana Márcia Suarez-Fontes, Juliana Almeida-Silva, Alessandra Lifsitch Viçosa, Sandra Aurora Chavez Perez, Alejandro Marcel Hasslocher-Moreno, Gabriel Parreiras Estolano da Silveira, Luciana Fernandes Portela and Roberto Magalhães Saraiva

Abstract

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is a major neglected disease endemic to Latin America, associated to significant morbimortality comprising a remarkable socioeconomic problem mainly for low-income tropical populations. The present chapter focuses translational research on Chagas disease, approaching drug combinations and repositioning, particularly exploiting the parasite oxidative stress by prospecting prooxidant compounds combined with antagonists of antioxidant systems, for developing low-cost and safe therapies for this infection. The pertinent literature on protozoal parasitic diseases is reviewed as well as on repurposing disulfiram aiming the combination with the Chagas disease drug of choice benznidazole. Both disulfiram and its first derivative sodium diethyldithiocarbamate (DETC) are able not only to inhibit p-glycoprotein, possibly reverting resistance phenotypes, but also to reduce toxicity of numerous other drugs, heavy metals, etc. Therefore, this innovation, presently in clinical research, may furnish a novel therapeutic for *T. cruzi* infections overcoming the adverse effects and refractory cases that impair the effectiveness of Chagas disease treatment.

Keywords: drug combination, drug repositioning, translational medicine, Chagas disease, oxidative stress, *Trypanosoma cruzi*

1. Introduction

Chagas disease (CD), the parasitic infection caused by the kinetoplastid protozoan *Trypanosoma cruzi*, is also known as American trypanosomiasis, for the huge endemic areas in South and Central Americas [1], but autochthonous human [2–4]

and domestic/wild animal [5–8] cases were reported in the United States, and due to migration, it is already considered a public health problem on a global scale reaching different continents [9–11]. It is noteworthy that climate changes may promote the northward insect vector propagation [12], possibly generating new foci or endemic areas, and suitable climatic conditions may be available in African and Asian nations [13]. Besides the vector bloodmeal, congenital, blood transfusion and organ transplantation [14], CD may be transmitted orally via food and beverages contaminated by triatomine feces such as sugarcane and açai juices [15, 16] and even water, stored in/near domiciles in arid regions [15, 16], as the parasite is able to survive in such media [17].

It is alarming that 6–7 million people are estimated to have CD worldwide, with *circa* 173,000 new cases/year and over 75 million people are at risk. CD is the parasitic disease of highest mortality in Latin America as 9490 deaths were reported in 2019. Furthermore, the real prevalence is largely unknown as most chronic patients are asymptomatic and even symptomatic patients have poor access to health public system. CD is endemic in 21 countries in Central and Latin America where about 5.7 million people have CD and 25% of the population is at risk [18]. In 2020, it was estimated that there were 3.2 million infected people, which can reach 1.5% of the general population. In addition, about 70 million are at risk of infection [19]. The prevalence of CD is presumably vastly underestimated. In January 2020, a study carried out by the ArtScience Initiative for Health Promotion, carried out by Oswaldo Cruz Foundation (Fiocruz) and collaborating organizations, showed a CD seropositivity of 20% in a tested population of an endemic area [20]. It must be mentioned this study was not designed to access CD prevalence and was biased by the population intention to get diagnosis procedures.

CD represents economic losses in excess of $1.2 billion/year to endemic countries in South America, in addition to more than $7 billion a year at global levels [21], including treatment and loss of productivity. Since no proven effective and approved vaccines are available for this disease, chemotherapy represents the only therapeutic intervention, as well as an important way to control them.

CD etiological treatment is directed according to the phase and clinical presentation of the disease, which is mandatory in the acute phase, congenital cases, or reactivation due to immunosuppression. In the chronic phase, the trypanocidal treatment is indicated in children and adolescents, recent infection, and women of childbearing age [22].

2. Therapeutics

Although CD was discovered and is studied for over a century [14], the etiologic treatment is still based on solely two drugs (**Figure 1**): the nitrofuran derivative nifurtimox (NFX; Lampit®, Bayer; 5-nitrofuran(3-methyl-4-(5′-nitrofurfurylideneamine)tetrahydro-4H-1,4-tiazine-1,1-dioxide), and the 2-nitromidazole benznidazole (BZ; LAFEPE; N-benzyl-2-nitroimidazole-acetamide) [23]. Both NFX and BZ were shown to produce remarkable ultrastructural alterations in mammal cells and tissues [24, 25], which were apparently more pronounced in NFX-treated animals [26]. Therefore, experimental chemotherapy studies approaching parasites as *T. cruzi* should preferentially include ultrastructural analysis, in order to offer a subcellular compartmentation understanding to aid the antiparasitic agent mechanism(s) of action elucidation [27, 28] and ultimately leading to the understanding of cell death pathways involved [29].

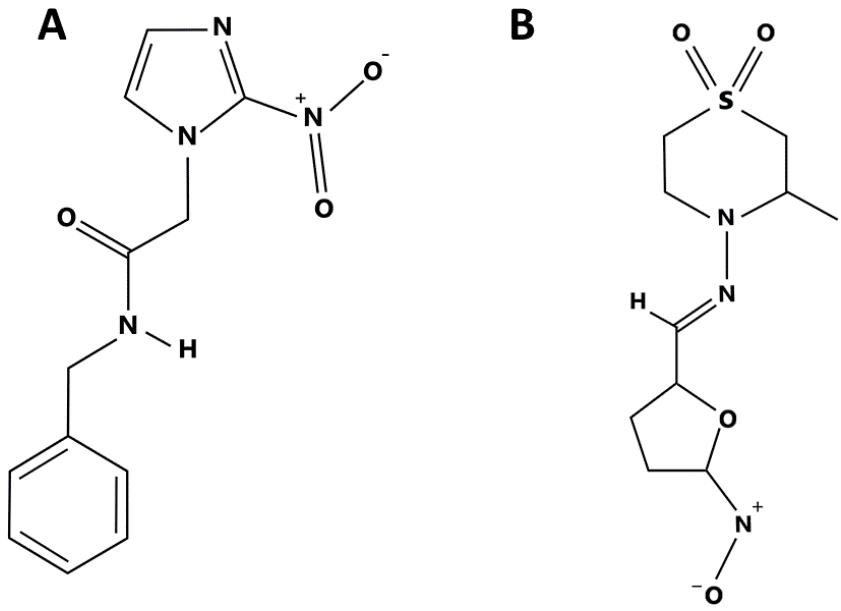

Figure 1.
Molecular structures of the nitroheterocyclic drugs employed in the treatment of Chagas disease: the 2-nitroimidazole benznidazole (A) and the 5-nitrofuran nifurtimox (B).

The CD therapeutics remain unsatisfactory, as they are associated with adverse effects [30–32], affecting 84.8 and 95.2% of patients treated with BZ and NFX, respectively [33], which may be severe, leading to the irreversible suspension of therapy in CD, in ≈20% [34, 35], ≈30% [36, 37], 41.5% [38], and up to 50% of the cases [39, 40]. Treatment suspension using NFX was reported in 43.8% of patients [33]. In an early study based on small samples, NFX was reported to be associated to definitive treatment interruption in 75% of patients [38]. Nevertheless, treatment intolerance was reported at similar levels with the use of the two drugs, approached by the same team [34, 35], but adverse effects, including neuropsychiatric events, may be more frequently associated to NFX [33]. In addition, it was reported that among patients who had discontinued BZ treatment and were treated with NFX, 12.3% also developed adverse effects that required definitive discontinuation of therapy [39]. Nevertheless, NFX was reported to be safe as a second-line therapy in patients who discontinued BZ [41].

Most CD patients are not treated because of the insufficient diagnosis and low cure rates observed in chronically infected patients [42], although treatment may diminish the disease progression and cardiovascular events [43, 44]. In addition, the CD treatment accomplishes only a parasitological cure, and a clinical cure is hardly proved [43, 45]. Whereas the *bona fide* sterile cure or complete clearance of the infection is considered a "prerequisite" for new anti-*T. cruzi* drug candidates [46], it is usually not achieved in murine model [47, 48] or human infection as immunosuppression often leads to infection reactivation [49]. In this regard, *T. cruzi* amastigotes may persist in a dormant or quiescent form, which may protect the parasites from antiparasitic agents [50, 51].

As the dormancy state of *T. cruzi* amastigotes is associated to drug resistance [50, 51], it is desirable to develop drugs able to affect dormant parasites. The mechanisms that allow the establishment of persistence include the capacity to suppress

the oxidative burst produced by phagocytes largely depending on iron-containing superoxide dismutases (FeSOD) and trypanothione-acting enzymes [52]. Thus the use of disulfiram (DSF) is of potential relevance since it can diminish glutathione levels [53, 54], and the DETC first derivative of DSF is an SOD inhibitor [55, 56]. Furthermore, DSF could target *T. cruzi* dormancy. Although the signal transduction pathways involved in this process were not completely elucidated, it is interesting that DSF is able to reverse HIV latency affecting PKC (protein kinase C), AKT (protein kinase B), PI3K (phosphoinositide 3-kinases), NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) [57, 58], which also affect *T. cruzi* infection [59, 60] that leads to the activation of PI3K [61], whereas DSF promotes PI3K inhibition [62].

An important study [63] approached the persistent parasite elimination, but the use of higher BZ doses might pose higher risks for patients. In this regard, the polyamine and thiol synthesis *Leishmania* are associated to macrophage M2 phenotype, leading to parasite persistence [64].

2.1 Drug resistance

Besides considerable severe adverse effects, one of the greatest problems of CD therapeutics is the selection of resistant parasites, impairing its effectivity, therefore causing refractory cases. BZ and NFX resistance is readily developed *in vitro* and *in vivo* [47, 65], in the former case, via different mechanisms that can act in concert [66].

Despite significant time and resources investments by innumerous research institutions over the world, only a few therapeutic candidates advanced the pipeline to treat neglected diseases such as CD [67]. It is alarming that it usually takes over 10 years to develop new drugs, whereas resistant parasites are rapidly selected. Also, there are naturally resistant *T. cruzi* strains [68–70] that express a novel ABCG-like transporter [71]. Besides extrusion pumps, *T. cruzi* resistance may involve SOD and trypanothione (*vide infra*). Therefore, there is pressing demand for the development of novel effective therapies for CD.

3. Oxidative stress in Chagas disease

Oxidative stress is a central phenomenon involved in aging, cancer, transmissible or infectious diseases, including COVID-19 [72], nontransmissible chronic conditions, such as metabolic diseases, autoimmune and degenerative disorders, inflammation, metal poisoning, etc. [73–75], produced by the imbalance on the production/uptake of oxidant/antioxidant species [76].

A plethora of antioxidant defenses evolved in order to balance the redox homeostasis [76, 77]. Oxidant species such as superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) are detoxified by SOD and catalase, respectively. Most cells rely also on the peptide glutathione (GSH), able to chelate reactive oxidant species (ROS) via cysteine sulfhydryl (SH) group and function as substrate for enzymes including GSH reductase and GSH peroxidase [78].

Although most of these processes are evolutionary conserved, some of the antioxidant defenses pathways differ between mammals and pathogens, therefore comprise potential chemotherapy targets. Contrary to mammals, GSH in trypanosomatid parasites mostly takes part in the adduct with the polyamine spermidine, forming

*N*1,*N*8-bis(glutathionyl)spermidine (trypanothione, TSH), and therefore its expression depends on the GSH, TSH [79], and polyamine [80] metabolism pathways.

Metabolomics and gene expression studies [81] reveal the participation of both GSH and the spermidine synthesis pathway, indicating the participation of trypanothione, in the regulation of redox metabolism in trypanosomatids. GSH is very relevant not only in oxi-reductive homeostasis, as this molecule is also related to detoxification and resistance to different drugs/xenobiotics in tumor cells [82, 83] binding to drugs that are extruded via multidrug resistance transporters [84]. TSH binding to NFX and BZ is involved in the detoxication of these trypanocides [85, 86]. Therefore, glutathione/trypanothione can promote the action/reverse resistance to different drugs. *T. cruzi* parasites overexpressing trypanothione synthetase tolerated higher doses of BZ and NFX [87]. Conversely, the GSH biosynthesis inhibition using buthionine sulfoximine increases the efficacy of NFX and BZ upon *T. cruzi in vitro* [88] and NFX *in vivo* [89] as well as of stibogluconate on *Leishmania* (*L.*) *donovani* [90].

Interestingly, polyamine play pivotal roles in parasite cells [91, 92], including *T. cruzi* antioxidant defense [93], and its synthesis and transport pathways provide valuable chemotherapy targets [94, 95], including repositioned drugs [96].

Parasitic diseases such as CD are correlated to oxidative stress [97, 98], associated to triggered chronic inflammatory reactions [99, 100]. Endogenous oxidative stress may be produced by cell organelles, mainly mitochondria [101, 102]. The CD myocarditis is characterized by intense oxidative stress due both to inflammatory response associated to neutrophils and macrophages NADPH oxidase (Nox) activity and the macrophage superoxide produced by Nox2 is required for parasite control in early infection [103]. The mitochondrial ROS produced by cardiomyocytes plays a relevant role in intracellular oxidative stress and inflammation, causing myocardium tissue damage [104–106]. These events are not independent since mitochondrial ROS may trigger proinflammatory cytokines via NFkB and PARP/PAR pathways [107], and the mitochondrial MnSOD activity may revert much of the inflammatory foci and necrosis [105], and ineffective antioxidant defense is associated to oxidative stress [108]. Exosome or extracellular vesicles liberation may also contribute to inflammation and oxidative stress [107, 109]. The oxidative stress is also involved in neurodegeneration in both cardiac and gastrointestinal tissues [110]. The chronic oxidative stress in the nervous tissue is associated to cognitive deficit, which can be reversed by BZ treatment [111].

Thus, the use of adjuvant antioxidant agents may ameliorate the cardiac pathogenesis [107, 112, 113]. Interestingly, vitamin C, widely considered antioxidant, can at high concentrations also function as a prooxidant, undergoing pH-dependent autoxidation, leading to H_2O_2 formation [114, 115]. In CD models, ascorbic acid can also reduce parasitemia, promote BZ action, and enhance animal survival in murine infection [116, 117].

ROS production comprises a well-known microbicidal immune effector mechanism [118]; therefore parasite borne antioxidant systems are not only virulence factors [119]. Besides the parasiticidal activity, ROS may function as signaling molecules promoting parasite proliferation. As in the Paracelsus adage, "The dose makes the poison" (Latin: *sola dosis facit venenum*), ROS in mammalian cells may trigger different responses depending on concentration. Low ROS levels may have signal transduction roles, inducing responses such as activation, proliferation, and differentiation, whereas at higher levels such molecules are generally cytotoxic, leading to

cell death [120]. Similarly, in *T. cruzi*, low ROS levels may signal for parasite invasion of host macrophages [121] and proliferation mainly in the acute phase [122], but high ROS levels culminate in programmed cell death, which may be inhibited by enhanced SOD expression [87]. Interestingly *T. cruzi* amastigotes undergo stress-induced proliferation [123].

4. Oxidative stress as a source of chemotherapy targets

Numerous therapeutic strategies exploit redox systems [124], including protozoal diseases [125], such as CD [126]. Therefore, antioxidant systems including SOD, trypanothione, and enzymes action on this glutathione-spermidine adduct (*N*1,*N*8-bis(glutathionyl)spermidine), such as trypanothione reductase, can comprise important chemotherapy targets [127]. Natural products such as the naphthoquinones α-/β-lapachones [128–130] and their derivatives [131, 132] have microbicidal activity against *T. cruzi*, among other pathogens [132]. Interestingly, β-lapachone derivatives were shown to cause mitochondrial dysfunction [131], damage [133], and autophagy, including mitophagy as well as apoptosis and necrosis [134]. In this regard, mitochondria comprise important therapeutical targets for cancer [135], aging [136], cardiovascular diseases [137], and degenerative diseases such as rheumatoid arthritis [138], Alzheimer's disease [139], Parkinson's disease [140], etc. Mitochondria are also promising target for antiparasitic [141, 142] and particularly antiprotozoal [143–145] therapeutic agents, specifically approached in trypanosomatids [146–148].

Up to 2% of the O_2 reaching the mitochondrial matrix is converted to $O_2^{\bullet-}$ (superoxide anions) forming H_2O_2 via SOD [149]. Like mammalian cells, *T. cruzi* mitochondria are a source of ROS [150] producing superoxide. Therefore, the Mn-SOD is important for controlling oxidative stress in this redox organelle. Contrary to mammals, the trypanosomatid mitochondria present FeSOD [151] that can protect from $O_2^{\bullet-}$ produced by macrophages [152].

Because of the prooxidant effects of antiparasitic drugs [126, 153–155], ROS detoxifying systems may comprise valuable scape mechanisms from pharmaceutical intervention [156] and programmed cell death triggered by mitochondrial $O_2^{\bullet-}$ [157].

The prooxidant capacity of both NFX and BZ, particularly in the former, is due to redox cycling with the production of $O_2^{\bullet-}$ [126, 158–160]. Superoxide may be not produced by BZ in the parasite, but in the host cell [161]. Therefore, FeSOD is linked to BZ resistance in *T. cruzi* [66, 162, 163]. Proteome of BZ-resistant *Trypanosoma cruzi* revealed enhanced FeSOD activity [164]. BZ resistance was associated to decreased cytosolic SOD but enhanced mitochondrial MnSOD and tryparedoxin-1 [165]. The deletion of the *sodb1* gene enhances *Trypanosoma brucei* susceptibility to BZ and NTX [166]. FeSOD is also implicated in drug resistance in *L.* (*Viannia*) *braziliensis* and *L.* (*Leishmania*) *infantum* [167, 168] *Entamoeba histolytica* [169]. Tryparedoxin peroxidase is also associated to antimony resistance in *L.* (*V.*) *braziliensis* [170]. In addition, SOD inhibition was reported to decrease parasitemia in *T. cruzi* murine infection [171].

Sirtuins are a highly conserved family of enzymes that deacetylate lysine residues on histone and non-histone proteins, using NAD^+ as a cosubstrate, regulating cellular antioxidant/Redox mechanisms [172, 173]. It is noteworthy that SIRT3, 4, and 5 are found in the mitochondrial matrix [174]. As cardiomyocyte mitochondrial dysfunction plays a central role in chagasic myocarditis (*vide supra*), the activation of sirtuins such as SIRT1 by agonists including resveratrol may enhance antioxidant defenses

[175], and SIRT3 activates MnSOD, scavenging ROS [176]. Nevertheless, the sirtuin TcSir2rp3 was shown to increase *T. cruzi* resistance to BZ and NTX for overexpressing TcFeSOD-A activities [177].

Selenium and selenium-containing compounds show beneficial effects both in murine [178–180] and human *T. cruzi* infection [181, 182], therefore comprise promising coadjuvant therapies for CD [183–185], although selenium was previously reported to increase tissue parasitism [186].

This activity maybe largely dependent on redox regulation as this inflammatory infection is associated with intense oxidative stress, and selenium may be antioxidant [187] and anti-inflammatory [188], as well as catalyze hydrogen peroxide (H_2O_2) reduction [189], therefore possibly diminishing the oxidative stress in infected cardiomyocytes, by impairing the Fenton reaction in the presence of iron.

5. Repositioning and combining drugs

The combination of different drugs may pose the advantage of supra-additive effects, which may be synergistic, in parasite models such as *T. cruzi* [190], *Plasmodium falciparum*, *Trypanosoma rhodesiense* [191]. The identification of synergistic combinations is relevant since they tend to present higher selective indices [192, 193], consequently, avoiding side effects and potentially permitting development of antiparasitic agents used at lower concentrations.

The identification of drug combinations with multiple targets can lead to the use of novel multitarget mechanisms able to cope with the challenge of multigenic diseases [194] and/or chronic infections with complex pathophysiology. It is noteworthy that the pharmaceutical properties of the combination may be absent in the components alone [195], generating the innovative concept or science field termed polypharmacology with numerous applications on drug repurposing [196] and CD [197]. As the philosopher Aristotle (384–322 B.C.) stated: "The whole is greater than the sum of its parts."[1]

Furthermore, drug combinations are largely employed for preventing drug resistance [198–204]. However, this strategy is not constantly successful as the reports of resistance to the sulfadoxine-pyrimethamine combination began in the same year this antimalarial regimen entered the clinic [205]. Similarly, the discovery of artemisinin (ART) costed Youyou Tu over 30 years of hard work [206] and was worthy a Nobel Prize, but *P. falciparum* resistance to the drug was detected after about 10 years of use [207]. The antimalarial combination therapies based on the use of ART were considered key to the elimination of malaria [208], but in the very same year [209, 210] and even earlier [211], the arteminisin derivatives combination therapy failures were reported. In the case of CD, the problem may be even more upsetting as natural resistance isolates are arising, particularly in the Amazon region (*vide supra*). Thus, effective strategies to prevent different mechanisms of drug resistance to arise are immediately needed.

Approaching repositioned drugs with available pharmacokinetic and toxicological properties can shorten the long and expensive path between *in vitro* trials and new drugs. While the period between drug discovery and approval can be 12–16 years at a

[1] "Since that which is compounded out of something so that the whole is one, not like a heap (…), then, is something-not only its elements (…) but also something else (…)" 'Metaphysics' Book VII by Aristotle, Translated by W. D. Ross, often misquoted or mistranslated.

cost of US$1–2 billion, repositioned drugs can enter the clinic in ½ the time, at *circa* 1/3 the cost [212], with much higher success rates [213].

Drug repositioning maybe a promising approach in CD [214–227]. Similarly, drug combinations may be instrumental in CD [197, 228–233], and both strategies may be employed and associated [214, 234–236]. Furthermore, drug combinations can increase success of drug repositioning [237]. In addition, it was accurately hypothesized that the combined use of repurposed drugs with BZ could be more efficacious than BZ alone [238].

5.1 Repositioning disulfiram

Disulfiram (DS, 1,1′-disulfanediylbis(N,N-diethylmethanethioamide) also termed tetraethylthiuram disulfide; CAS no. 97-77-8; Molecular Formula: $C_{10}H_{20}N_2S_4$), a repositioned drug used in alcoholism and marketed as Antabuse® (**Figure 2**), was approved for medical use over 70 years ago and is widely used since then [239, 240].

At the very beginning, the discovery of thiocarbamates and its derivatives was serendipitous and showed clear signs of versatile perspectives that unequivocally culminated in the present promising repurposing strategies for both pharmaceutical and industrial applications [241, 242].

In the 1930s and 1940s, dithiocarbamates such as dimethyldithiocarbamates and diethyldithiocarbamates were used as pesticides against fungal pathogens on different crops [243], besides biocides in household products [244].

The industry plant physician E. E. Williams in 1937 observed that workers using tetramethylthiuram monosulfide and disulfide to facilitate the rubber vulcanization became alcohol-intolerant and quit consuming alcoholic beverages. The DSF-induced alcohol aversion was described in 1948 [245]. At that time, DSF was approached as a vermicide and employed as an ointment to treat scabies.

Afterward, besides alcoholism, DSF started to be studied for heavy metal poisoning, cancer [246–249], HIV [243, 250], as well as cocaine dependence, pathological gambling, and other psychiatric disorders [239] and other form of addiction, for example, the d-methamphetamine abuse [251]. Further tests are being performed focusing applications such as Alzheimer's disease [252], Lyme disease and babesiosis [253], tuberculosis [254], non-tuberculous mycobacteria infections [255], giardiasis [256], amoebiasis [257], obesity [258] and to revert drug resistance in different types of cancer [259–261], tuberculosis [262] bacterial infections [263], mycosis [264], giardiasis [265], etc. The repositioning of low-cost drugs such as DS is considered a "salvation" for global healthcare system [266].

Sodium diethylcarbamodithioate (**Figure 2**) (DETC also known as sodium (diethylcarbamothioyl)sulfanide; CAS no. 148-18-5; Molecular Formula: $C_5H_{11}NS_2.Na$) is the first derivative of DSF, involved in many of the biological activities of the latter.

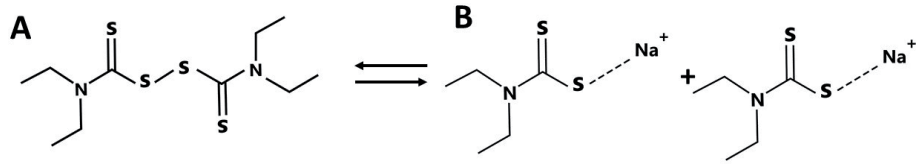

Figure 2.
Molecular structures of disulfiram (A) and sodium diethyldithiocarbamate (B).

Seemingly DETC is less toxic than aspirin [243], widely used, and well tolerated in humans [267] for decades being used up to 800 mg/twice/week, with no adverse effects [268]. DETC also known as Imuthiol or Dithiocarb was used as immunomodulator with good results on AIDS patients [269, 270] and was clinically employed in chronic bronchitis, rheumatoid arthritis, tuberculosis, and chronic infection [271].

In a seminal report on its antiparasitic activity, DETC was demonstrated to be leishmanicidal [272]. Afterward, novel delivery systems were developed to optimize the leishmanicidal activity of DETC [273–275]. In this regard, novel drug delivery systems are also developed for DSF [276]. The data obtained on Leishmania amazonensis motivated us to move to CD, employing the repositioned drug DSF combined to the drug of first choice BZ. Tests on NFX are in progress.

It is worth remembering that CD pathophysiology is associated with oxidative stress (*vide supra*), and both DSF [277] and DETC [278] can act as antioxidants. In addition, modulation of oxidative stress comprises a valuable tool in heart disease therapeutics [279]. In addition, DSF has antimutagenic properties [280].

5.2 Disulfiram combined to benznidazole in Chagas disease

Both DSF and DETC have antiparasitic activity on *T. cruzi* [281, 282], but the effectivity was not pronounced.

In our study, the DSF-BZ combination is promising since the antagonism of SOD activity can enhance oxidative stress in cancer cells [249] and *T. cruzi* [283]. In this regard, the antitumor activity of NTX is enhanced by SOD1 inhibition mediated by tetrathiomolybdate [284]. Both in vitro and in vivo experimental data confirmed the present assumption [Almeida-Silva et al., in press]. The SOD inhibition as well as TSH reaction by DSF/DETC can promote the intracellular accumulation of ROS leading to parasite death (**Figure 3**).

CD etiological therapy is often associated to severe adverse effects caused by the highly toxic drugs (*vide supra*). In this sense, the present innovation involves the advantage of employing DSF/DETC with cytoprotective properties [243] in different cell types.

DSF/DETC have neuroprotective [285], hepatoprotective [277], and nephroprotective [286] and even radioprotective [287, 288] activity. These protective effects may be beneficial in the treatment of parasitic diseases, because in the treatment of experimental infection by *T. rhodesiense*, DSF has marked protective activity (disulfiram rescue) against the toxic effects of diaminodichloroplatin and preventing the death of the treated organism [289].

Thus, the development of low-toxicity therapies may be expected, as DSF may have a protective action against the toxic effects of drugs such as cyclophosphamide [290], ifosfamide [291], N-nitrosodimethylamine [292], isoniazid [293] and the toxicity of α-naphthylisothiocyanate [294], acetaminophen [295], pyrrolizidines [296], the lethal effects of hypoxia [297], ischemia [298], as well as lead [299], cadmium [300], mercury, and other heavy metals [301]. Thus, DSF combinations can enable the development of safe medicines. Regarding CD, the cardioprotective and antioxidant activities of DSF/DETC as well as atrial neuroprotection [302] are particularly desirable [303–306]. In addition, DSF is effective as prophylactics in experimental colitis [307].

As drug resistance limits the successful CD therapy, the *T. cruzi* PgP expression has a pivotal role [308]. Therefore, it is relevant in the present approach that DSF/DETC inhibit PgP [261, 309, 310], causing the BZ accumulation within the parasite

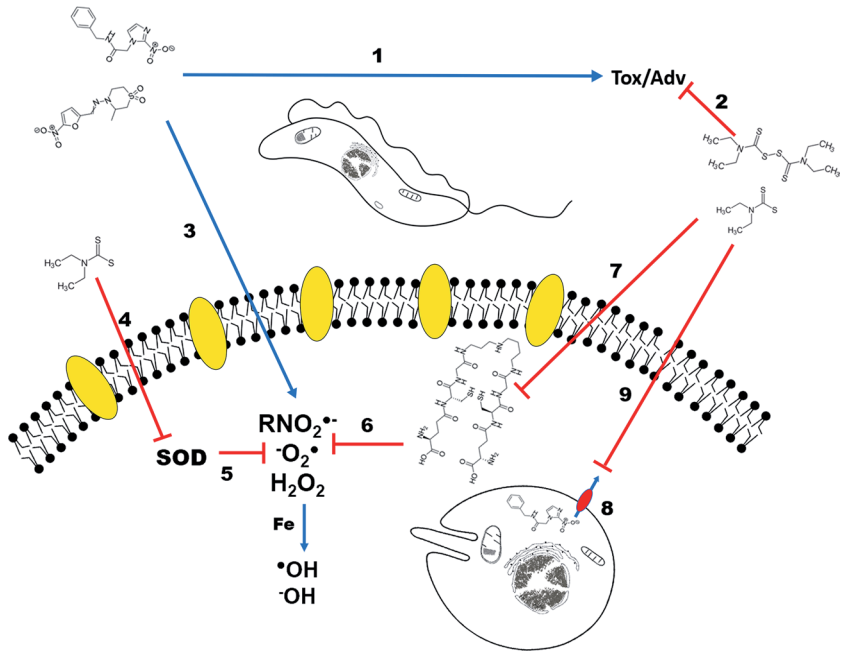

Figure 3.
*Putative mechanisms of action of disulfiram (DSF) or diethyldithiocarbamate (DETC) in combination with trypanocides in T. cruzi infection. Benznidazole (BZ) and nifurtimox are toxic and produce adverse reactions (**1**), which are ameliorated via detoxification mediated by DSF or DETC (**2**). The anti-T. cruzi agents trigger the formation of reactive oxygen species (ROS, **3**) via nitroanion radicals ($RNO_2^{\bullet -}$) that give rise to superoxide ($O_2^{\bullet -}$), that is detoxified by superoxide dismutase (SOD, **5**), generating hydrogen peroxide (H_2O_2), which in the presence of iron can produce hydroxyl radicals ($^{\bullet}OH$) and hydroxide anions (^{-}OH) via Fenton reaction. DETC inhibits SOD (**4**). ROS may be detoxified by reaction with sulfhydryl or thiol groups of trypanothione (N1,N8-bis(glutathionyl)spermidine, **6**), and this adduct can be removed by reaction with thiols of DSF/DETC (**7**). The BZ molecules in the parasite cytoplasm are extruded from the cell via p-glycoproteins or MDR transporters (**8**), which are inhibited by DETC (**9**), presumably reversing resistance phenotypes.*

cytoplasm, enhancing trypanocidal activity, potentially reversing resistance phenotypes, such as MDR^+ (**Figure 3**). Interestingly, the ABCC proteins from *T. cruzi* are involved in thiol transport [311]. In view of the glutathione-drug adduct transport by ABC transporters (*vide supra*), it is interesting that DSF reduces GSH levels [54] at least in part through the formation of complexes with its different derivatives [312].

DSF [313] affects the redox balance of the cell, to GSH oxidation [314], reducing GSH levels [54] at least in part through the formation of complexes with its different derivatives [312, 315]. DETC can also reduce the GSH/non-protein thiol levels, also leading to the reduction of glutathione peroxidase activities [53, 316].

The combinations tested here may also contribute to resistance reversal, also through DETC-mediated inhibition of Fe-dependent SOD, which is linked to resistance to BZ in *T. cruzi* [66, 162, 163].

Furthermore, DSF can be used against cancer cells targeting the ubiquitin-proteasome system [317], and the ubiquitin-proteasome pathway is a therapeutic target in *T. cruzi* [318].

In this way, the strategy based of combinations of the repositioned drugs proposed here can achieve effectiveness, with selectivity and, therefore, safety in the CD treatment and sheds new light on perspectives for new therapeutic strategies.

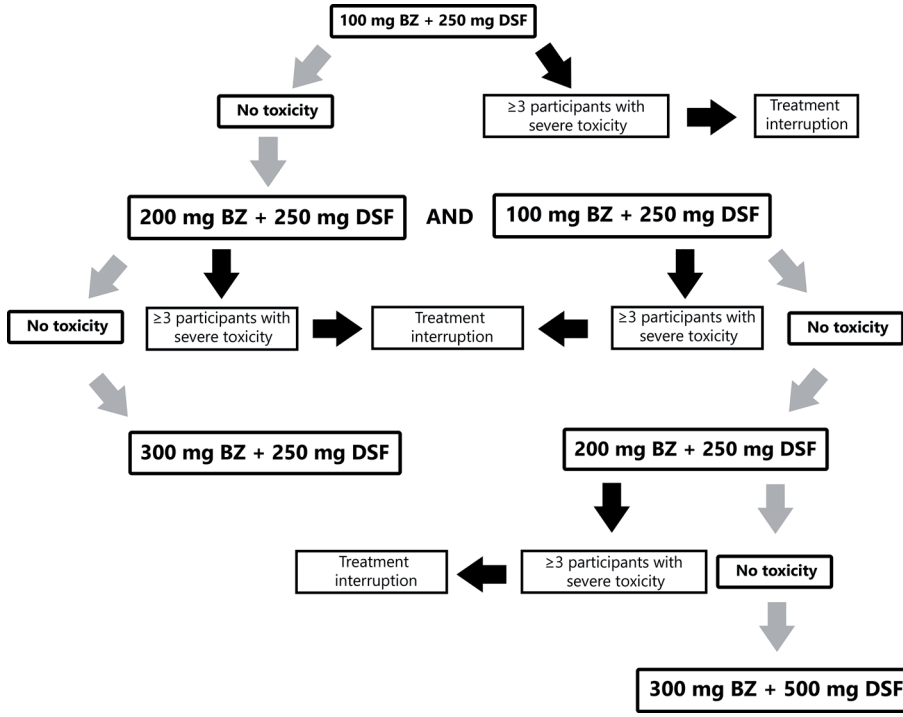

Figure 4.
Design of the clinical trial for testing the BZ-DSF combination. Reproduced from Ref. [320] (with permission).

6. The clinical stage

Translational research in biomedical sciences translates basic research and experimental discoveries into health taking the route from benchtop to bedside. This important field has gained substantial attention and investments in the last two decades [319].

In order to reach a proof of concept on the effectivity of the DSF-BZ combination in human infection, a partnership was established gathering different units of Fiocruz. The present study comprises a translational approach that began with experiments in vitro, on the bench and now reaches the clinical stage at the Evandro Chagas National Institute of Infectious Diseases-Fiocruz, coordinated by the team of the Clinical Research Laboratory of Chagas Disease, with assistance of the Clinical Research platform. Therefore, the phase I/II clinical trial was elaborated (**Figure 4**) and published recently [320].

7. Conclusions and future perspectives

The use of DSF/DETC combined to BZ in CD treatment comprises a potential innovative therapeutical tool, possibly overcoming adverse reactions and refractory cases. Since these repositioned drugs exert cytoprotective effects, reducing the adverse reactions of many drugs, safe combinations can be potentially identified, leading to the development of well-tolerated medication. Therefore, therapy interruption can be precluded, consequently increasing patient adherence. In addition,

as DSF/DETC can inhibit p-glycoprotein activity as well as reduce GSH levels, two molecules involved in drug extrusion from MDR^+ parasites, it is reasonable to suppose the combination could eventually revert/downmodulate natural/acquired resistance phenotypes. Thus, treatment may be effective even in refractory cases. We are now approaching the clinical response of chronic phase CD patients. A possible proof of concept may lead to the development of a safe and effective medication, with profound implications in treatment prognosis, presumably improving the quality of life of the patients.

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